Anatomical study of the variation in the branching patterns and histology of the aorta in a South African population

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Declaration

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I dedicate this dissertation to my late Ouma,

Helena Dorothea Petronella da Silva.

Thank you for your gentle soul and the part it played in my upbringing.

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Abstract

Background

The documentation of variations in branching patterns of the aorta among South African populations is limited. Histological changes in the aortic wall have been documented and may be due to pathology and physiological processes. Whether these changes are solely due to physiological processes such as haemodynamics or pathology is yet to be determined.

Aim

The present study aims to document the branching patterns of the aorta in a South African population and to distinguishing which particular histological changes in this vessel wall can be associated with haemodynamic forces rather than pathology.

Methods

Seventy one cadavers from the University of Cape Town MBChB programme were used to document the branching pattern of the aorta. Twenty five random complete aortae with no evidence of macroscopic pathology were collected from Salt River Mortuary and used for histological examination. Seven sections (taken from branching and non-branching sites along the aorta) from each sample were stained with haematoxylin and eosin, alcian blue pH2.5 - periodic Schiff reaction and elastin von Gieson's for histological analysis using a Zeiss Axioskop Mot upright microscope and Axiovision 4.7 software.

Results

Variation in the branching pattern of vessels along the length of the aorta was found in 49% of the cadaver sample. A variety of histological variations of the wall of the aorta in the mortuary sample was noted. Noteworthy variations include the abundance of acid mucopolysaccharide, the increased thickness of the tunica intima, and increased elastin fragmentation of the tunica media at branching sites of the aorta. A pronounced curved ridge-like structure was indentation on the luminal surface at the junction of the arch and descending aorta in 74% of the mortuary sample. The presence of this aortic ridge was association with younger aged individuals (Chi-square 4.57, p=0.56).

Conclusion

A high frequency of gross variation in branching patterns of the aorta is present in this cadaver sample, including some rare patterns of variation. Clinically, knowledge of these variations would be relevant and useful to anatomists, radiologists and head, neck, thoracic and vascular surgeons. An explanation for the abundance of acid mucopolysaccharides may be the link between acid mucopolysaccharides and diet, however this needs further investigation. Altered haemodynamic forces created by turbulent flow at branching sites is proposed as the explanation for the increase in the thickness of the tunica intima and increased elastin fragmentation at the branching sites of the aorta, although other factors such as the effects of HIV in the vessel wall of this sample are yet to be determined. This study proposes that the aortic ridge is a result of the closure of the ductus arteriosus.

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Abbreviation of terms

AB-PAS	Alcian Blue pH2.5 - Periodic Schiff reactions
BMI	Body mass index
CVD	Cardiovascular disease
CVS	Cardiovascular system
EVG	Elastin von Gieson's
H&E	Haematoxylin and Eosin
HIV	Human Immunodeficiency Virus
MRC	Medical Research Council
UCT	University of Cape Town
WHO	World Health Organization

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So many people have been directly or indirectly involved in my journey of acquiring my Masters Degree. It has been a rollercoaster ride, but the following people (in no particular order) have made it manageable, but more importantly, enjoyable as well!

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Chapter 1: Introduction

1.1 Cardiovascular system

The circulatory system of humans comprises two components: the cardiovascular system (CVS) and the lymphatic system. The main functions of the circulatory system are to distribute oxygen and nutrients to all body tissues and to transport carbon dioxide and metabolic waste products from the tissues to the lungs and other excretory organs. Additional functions include distribution of water, electrolytes and hormones throughout the body, contribution to the infrastructure of the immune system, and thermoregulation (Aaronson, 2004).

The anatomical structures of the CVS include the heart, lungs, blood vessels, blood, lymphatic vessels and lymph. The lymphatic system is an open circulatory system with its main functions including drainage of interstitial fluid (lymph) from the tissue spaces, transportation of lymph back to the venous system, absorption and transfer of fat, and facilitation of the body's defence mechanisms (Moore et al., 2010).

The CVS is a closed system which consists of the heart, a network of arteries (the arterial system) and a network of veins (the venous system). The arterial system provides a distribution network to the peripheral microcirculation, which consists of capillaries and postcapillary venules and is the site where interchange of gas and metabolites takes place between body tissues and the blood. The three main types of vessels in the arterial system are elastic arteries, muscular arteries and arterioles (Young et al., 2007). The aorta is the largest (elastic) artery in the arterial system and transports oxygenated blood to the entire body. Descriptively, the aorta is typically divided into an ascending, arch, descending thoracic and abdominal segments. Along its length, the aorta gives rise to a number of named branches (Figure 1 for the typical branching pattern of the aorta as described in the literature) that supply oxygenated blood to specific regions of the body. Anatomical variations in the patterns of these branches are known to occur, however knowledge of these patterns is limited for South African populations.

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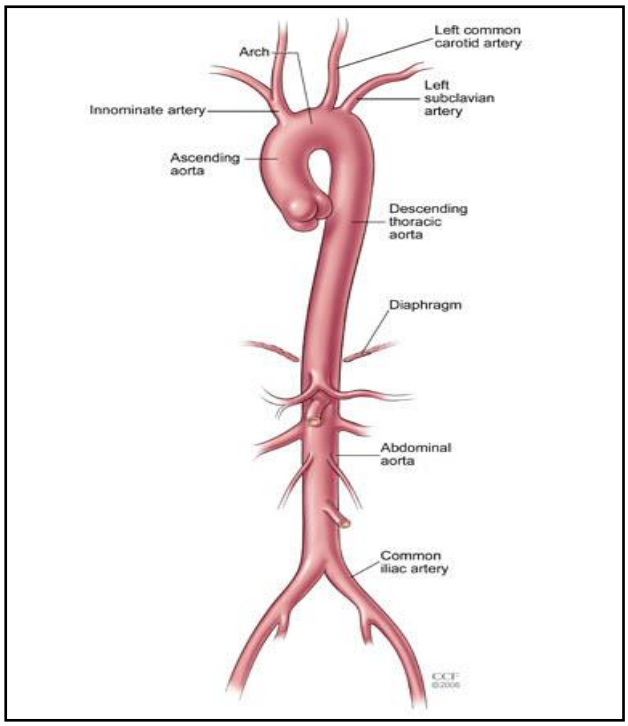

Figure 1.1: Diagram of the aorta and its named parts and branches (Note: Innominate artery will be referred to as brachiocephalic artery)

(Image from https://www.clevelandclinic.org/heartcenter/images/guide/disease/marfan/aortaLG.jpg)

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Histologically the general structure of arteries is similar throughout the body, although all vessels have structural specializations that relate to pulse pressure differences and the need to regulate flow of blood to tissues. Arteries and veins each comprise three layers or tunics which include an innermost tunica intima, a tunica media and an outer tunica adventitia (Figure 1.02).

The tunica intima comprises an endothelium lining the lumen of the vessel, resting on a supporting basement membrane. The polygonal endothelial cells are arranged on a network of collagen, elastic fibres, scattered fibroblasts, and deeper longitudinally orientated smooth muscle cells and macrophages (Virmani et al., 1991). The tunica intima is thickest in the elastic arteries.

The tunica media comprises smooth muscle cells and concentrically arranged layers or lamellae of elastic tissue, interspersed with collagen fibres. Relative deposits of muscle and elastic tissue vary according to the functions of the vessel, but the tunica media of arteries is

typically thicker than that of veins due to the higher blood pressures in the arterial system (Fawcett & Jensh, 2002).

The tunica adventitia comprises mainly of supporting connective tissue such as collagen fibres together with some elastic fibres and macrophages. This layer is relatively larger in veins than in arteries (Fawcett & Jensh, 2002).

Other features of blood vessels include the internal and external elastic laminae, comprising of elastin which provide both structural and functional support as well as separates the three tunics. These are present in both elastic and muscular arteries. The vasa vasora, which are small arteries that supply the walls of major arteries, can be found in the tunica adventitia of large elastic arteries.

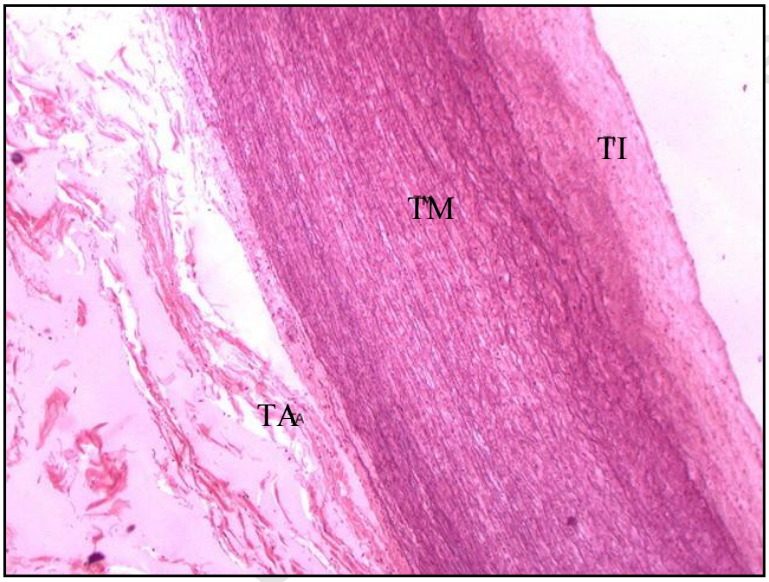

Figure 1.2: Histological features of an elastic artery (Haematoxylin and Eosin (H&E))
TI: Tunica intima, TM: Tunica media, TA: Tunica adventitia
(Image from http://bcrc.bio.umass.edu/bestofhistology/content/elastic-artery-s2009)

1.2 Cardiovascular disease

Cardiovascular diseases (CVD) are a group of disorders that affect the heart and blood vessels, and include coronary heart disease, cerebrovascular disease, peripheral arterial disease, rheumatic heart disease, congenital heart disease and deep vein thrombosis and pulmonary embolism (WHO, 2012a).

Cardiovascular diseases are now a leading cause of death, with over 17.3 million deaths from CVD in 2008, representing 30% of all deaths globally. It is estimated that 51% of these deaths are as a result of strokes and 45% as a result of coronary heart disease (WHO, 2012a). Both stroke and coronary heart disease can result from atherosclerotic arterial diseases which have become the leading cause of death in the CVD group (Alpert, 2012). In South Africa it is estimated that 76 252 people (13.7%) died from CVD in the year 2000 (Bradshaw et al., 2007).

1.3 Background

1.3.1 Prevalence of macroscopic arterial disease in the South African population

The majority of population-based studies on cardiovascular pathology, particularly peripheral arterial disease, have been based on White racial groups (Bennett et al., 2009). Far fewer studies have included the Black and Coloured South African racial populations. Of those studies that have focused on Black South African populations, all reported that peripheral arterial disease is a clinical problem that appears to be on the increase (Robbs, 1985; Madiba et al., 1999; Kumar Paul et al., 2007). Please note that the racial categories used in this study are those designated by the South African Police Services: Black refers to individuals of indigenous African ancestry; White to individuals of European ancestry; and Coloured to individuals of mixed (Khoi San, Indian, Indonesian, Black African and European) ancestry.

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In 2006, Franks conducted a pilot study on the prevalence of macroscopic atherosclerotic lesions in a South African mortuary sample. The results of this study indicated high levels of advanced macroscopic vascular pathology in Black African and Coloured males under the age of 50 years.

1.3.2 Prevalence of microscopic arterial disease in the South African population

The existence and prevalence of microscopic cardiovascular pathologies in the South African population are poorly documented in the literature. The few reports that do exist, report primarily on aortic aneurysm subsequent to intimomedial mucoid degeneration. Abdool-Carrim et al., (1996) found that intimomedial mucoid degeneration, a rare disease,

predominantly affects younger African females (average age of 52 years, ranging from 20-70 years old) with hypertension. In a 1955 study on Black South Africans, Pepler reported varying degrees of metachromatic mucoid degeneration in the tunica intima and tunica media of the aortic wall in all age groups. This degeneration was closely associated with the breakdown of elastic tissue as a consequence of the high levels of hypertension found in this population.

In a more recent study Van Kets et al., (2011) found that one third of a Cape Town mortuary sample population of 150 individuals showed evidence of both microscopic and macroscopic vascular pathology. The majority of the population with pathology were Coloured individuals under the age of 50 years. There are high levels of methamphetamine consumption among Coloured males (Darke et al., 2008; Plüddemann et al., 2009; Wechsberg et al., 2010), high levels of Human Immunodeficiency Virus (HIV) infection among young South Africans (WHO, 2012b), and genetically inherited hypercholesterolaemia in the population (Steyn et al., 1987; Kotze et al., 1995), all of which have been associated with atherosclerosis. It was suggested by Van Kets et al. 2011 that this could account for the high levels of vascular pathology. However these problems are not unique to South Africa (Ellis et al., 2007; Degenhardt et al., 2008), and with evidence that atherosclerotic lesions are found in the early stages of life (Fawcett & Jensh, 2002) it is suggested that the observed histological changes could be physiological (possibly due to haemodynamic forces) rather than pathological in nature.

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1.4 Justification for further study

The widespread presence of microscopic pathology of the aortic vessel wall across all age and racial categories and in macroscopically normal individuals described by Van Kets et al. (2011) certainly needs further investigation. The documentation of microscopic changes similar to those of atherosclerotic lesions in young individuals, including babies, without macroscopic evidence of atherosclerosis, suggests that some of the changes observed in the vessel wall in the South African population may be due to normal physiological processes rather than pathology.

Physiological forces at branching sites along arterial vessels have been noted to promote atherogenesis (Zarins et al., 1983; Asakuru & Karino, 1990; Glagov et al., 1992; Giddens et al., 1993).

Therefore further research is first needed to document the branching patterns of the aorta to see if there are any differences from other populations and whether this difference in anatomy, if present, could have any physiological implications. Physiological processes, such as haemodynamic forces associated with blood flow at the branching points of the aorta, could be possible explanations for the histological features seen in the aortae of the South African population.

1.5 Research Aims

The aims of this current study are to document:

1. The variation of the branching pattern of the aorta in a South African cadaver population
2. The histological structure of selected regions of the aorta and its main branches, subjected to varying haemodynamic forces, in a South African mortuary sample in order to determine which particular histological features can be associated with haemodynamic forces.

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Chapter 2: Literature review

This chapter describes the most common and classically termed normal branching patterns and morphology of the aorta. The chapter then looks at the development of the CVS as a source of the variation reported, and lastly comments on the documented variation in the branching patterns of the human aorta, and the documented histological variation of the human arterial wall.

PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) was used on the World Wide Web to search for electronic journal articles on these topics. Articles were sourced through the University of Cape Town (UCT) Faculty of Health Sciences library website (http://www.lib.uct.ac.za/medical/). Hard copies of the journal articles were obtained from the Health Sciences library, where possible, alternatively electronic copies were downloaded and saved. Text books were provided by the Health Sciences library and staff in the Department of Human Biology, UCT.

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2.1 Introduction

The aorta is the largest blood vessel in the body which begins at the aortic orifice of the left ventricle of the heart. It ascends as the ascending aorta in the superior mediastinum approximately 5cm to the sternal angle where it then becomes the arch of the aorta. The first branches of the aorta are the right and left coronary arteries of the ascending aorta which supply the myocardium and epicardium of the heart (Standring et al., 2006).

The arch of the aorta continues from the ascending aorta in the superior mediastinum and arches posteriorly on the left side of the trachea and oesophagus and superior to the left main bronchus. In approximately 65% of individuals the arch of the aorta has three branches: the brachiocephalic trunk (traditionally termed the innominate), the left common carotid artery, and the left subclavian artery. This pattern of branching is classically termed the norm in standard anatomy texts (Moore et al., 2010).

The descending thoracic aorta is a continuation from the aortic arch. It descends in the posterior mediastinum to the left of the vertebral column gradually shifting to lie in the midline at the aortic hiatus of the diaphragm (Standring et al., 2006; Moore et al., 2010).

Branches of the descending thoracic aorta include paired posterior intercostal, subcostal arteries, and superior phrenic arteries as well as multiple anterior visceral branches (e.g., bronchial, mediastinal, oesophageal and pericardial).

The abdominal aorta begins at the aortic hiatus of the diaphragm at the level of the T12 vertebra and descends approximately 13cm till it bifurcates into the right and left common iliac arteries at the level of the L4 vertebra. The most common branches of the abdominal aorta may be described as visceral or parietal and paired or unpaired. The unpaired visceral branches originate on the anterior aspect of the abdominal aorta and include the: coeliac trunk, superior mesenteric artery, and inferior mesenteric artery. The paired visceral branches originate on the lateral aspects of the abdominal aorta and are the: suprarenal arteries, renal arteries, and gonadal arteries (ovarian or testicular). The paired parietal branches originate on the lateral aspects of the abdominal aorta and include the: subcostal arteries, inferior phrenic arteries, and lumbar arteries. The unpaired parietal branch is the median sacral artery which arises from the posterior aspect of the abdominal aorta at its bifurcation (Williams et al., 1995; Moore et al., 2010).

2.2 Embryological development of the cardiovascular system

Variations in the branching pattern of the aortic arch have been reported in a large number of possible combinations with varying frequencies which may be related to the complex process of development of the aortic arch (Barry, 1951; Satti et al., 2007). In order to understand how these variations transpire, the embryonic development of the cardiovascular system must be addressed.

The cardiovascular system is one of the first systems to develop in the embryo due to the embryo's need to transport oxygen. Initial components of the cardiovascular system appear as angiogenic cell clusters in the extra-embryonic mesoderm lining the yolk sac (Mitchell & Sharma, 2005). These angiogenic clusters merge to form the cardiogenic area of the embryo, which subsequently forms the paired heart tubes. Two dorsal aortae develop on either side of the midline and connect to these heart tubes. Arteries throughout the body arise from the embryonic aortic arch complex, which includes six pairs of symmetrical aortic arches and the paired symmetrical dorsal aortae. The paired dorsal aortae join to form the descending aorta (Mitchell & Sharma, 2005).

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The embryonic aortic arches develop in a craniocaudal sequence, with the cranial arches disappearing before the caudal arches are completely developed. These arches undergo selective apoptosis and as a result the symmetrical arrangement of the vessels rapidly changes into the asymmetrical pattern that is observed in neonates and later in adults (Figure 2.1).

The 1st and 2nd aortic arches (I and II) regress completely. The paired 3rd arches (III) form the 1st part of the internal carotid arteries bilaterally. The proximal right 4th arch (IV) artery persists as the right subclavian artery to the origin of the internal mammary artery, while the distal right 4th arch artery regresses. The left 4th arch (IV) regresses and forms a small segment of the adult arch between the origin of the left common carotid and the left subclavian arteries. Little is understood about the 5th arch (V), but it seems it either regresses or is incompletely formed. The 6th arch (VI) is associated with the developing lung bud and forms the pulmonary arch, which develops into the right pulmonary artery and ductus arteriosus (Mitchell & Sharma, 2005; Standring et al., 2006; Satti et al., 2007). The result is that the residual vessels from the apoptosis process form the aortic arch and great vessels. During this process, anatomical variants can form (Satti et al., 2007).

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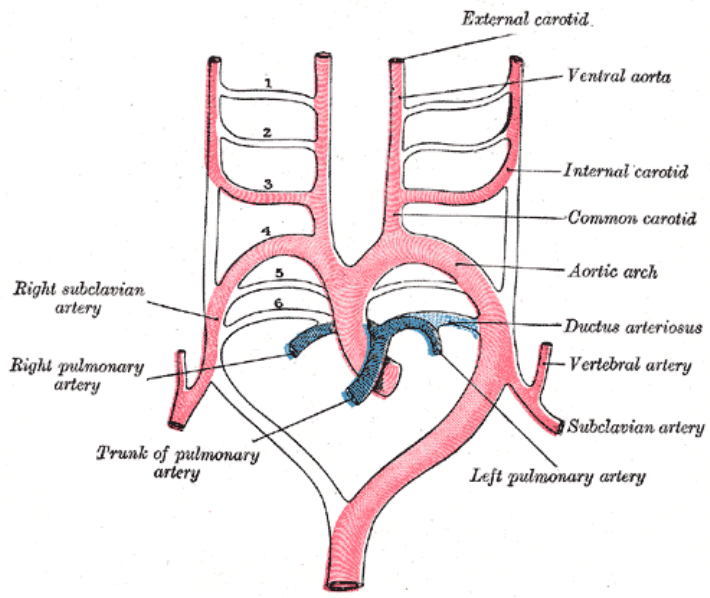

Figure 2.1: Diagram of the embryonic aortic arches
(1: Internal carotid artery, 2: External carotid artery, 3: Common carotid artery, 4: Right subclavian artery, 5: Arch of aorta, 6: Brachiocephalic artery, 7: Ductus arteriosus, 8: 7th intersegmental artery, 9: Pulmonary artery, 10: Carotid duct, 11: Obliterated right dorsal aorta
(Image from http://en.wikipedia.org/wiki/File:Gray473.png)

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2.3 Variation in the branching pattern of the human aorta

For the purpose of this review, the variation in branching patterns along the length of the aorta will be divided into aortic arch variation and variation along the descending aorta. Before these sections are discussed the variations of the coronary arteries need to be addressed. Variations in the branching patterns of the coronary arteries are common (Moore et al., 2010). In most individuals the right and left coronary arteries share the blood supply to the heart approximately equally. In roughly 15% of individuals the left coronary artery is dominant in that the posterior interventricular branch is a branch of the circumflex artery. Other variations include a single coronary artery, and a circumflex branch arising from the right aortic sinus. Rarely there is an accessory coronary artery (Williams et al., 1995).

2.3.1 Variation in the branching pattern of the aortic arch

The most common pattern of branching from the aortic arch, and classically termed the norm in standard anatomical text books, comprises three major arteries arising from the superior

aspect of the aortic arch. These branches are; the brachiocephalic trunk, the left common carotid artery, and the left subclavian artery. This pattern is present in approximately 65-80% of individuals (Bergman et al., 1988; Natsis et al., 2009; Bhattarai & Poudel, 2010; Jakanani & Adair, 2010; Shiva Kumar et al., 2010; Patil et al., 2012), but has been reported in as many as 94% of individuals by Nelson & Sparks (2001).

The second most common branching pattern of the aortic arch reported in the literature comprises two branches originating from the aortic arch and is referred to as a common brachiocephalic trunk. The common brachiocephalic trunk incorporates the brachiocephalic trunk and the left common carotid artery (Figure 4.4). A common brachiocephalic trunk has been described in numerous case reports in the literature, yet only a few studies have noted the prevalence of this variation in various samples. Nelson and Sparks (2001) examined the branching pattern of 193 human aortic arches in Japanese-American men as part of an epidemiologic study of stroke and vascular disease, known as the Honolulu Heart Study. These authors found a common brachiocephalic trunk in only 1% of their sample, although Moskowitz & Topaz (2003) reported a frequency of 3.2% and Adachi (1928) reported a finding of 11%. Moskowitz & Topaz (2003) investigated 1480 catheterized paediatric cases, while Adachi (1928) investigated hundreds of post-mortem bodies in Japan (the reference does not give an exact number).
These studies indicate that there is a wide range of variation in the prevalence of the common brachiocephalic trunk.

An additional variation in the branching pattern of the aortic arch includes the origin of a left vertebral artery arising from the arch as a separate fourth branch (Figures 4.2 & 4.3). The vertebral artery is normally a branch of the subclavian artery. Anomalous origin of the vertebral arteries is not common (Lemke et al., 1999). The prevalence of a left vertebral artery arising from the aortic arch has been reported as 1.5% (Nizanowski et al., 1982), 1-2.5 % (Daseler & Anson, 1959), and 5 % (Voster et al., 1998). The clinical significance of the anomalous origin and course of a left vertebral artery has been documented in cerebral disorders (Bernardi & Deton, 1975), and in head and neck surgery, angiography and arterial dissection (Vicko et al., 1999; Komiyana et al., 2001).

Rarer variations in branching patterns of the aortic arch include both a right and left common brachiocephalic trunk, and a retro-oesophageal right subclavian artery, amongst others. In

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the case of right and left common brachiocephalic trunks, only two branches arise from the aortic arch. Each brachiocephalic trunk incorporates the common carotid and subclavian artery of each side (left and right). This has been reported in 1.2% of individuals (Anson, 1963, as cited in Paraskevas et al., 2008).

A right subclavian artery branching as a fourth branch on the posterior aspect of the aortic arch passing towards the right axilla posterior to the oesophagus is known as a retro-oesophageal right subclavian artery (Figures 5.6 & 5.7). This rare variant has been well documented in the literature in the form of case reports due to its clinical relevance. *Dysphagia lusoria* or difficulty in swallowing and less frequently difficulty in breathing have been reported, particularly when the retro-oesophageal right subclavian vessel wall is calcified (Jebara et al., 1995; González-Panizo-Tamargo et al., 2011; Debonnaire et al., 2012; Derbel et al., 2012). Bergman et al. (1988) noted that this variation occurred in 0.4-2% of cadaver and radiographic samples.

2.3.2 Variation in the branching pattern of the descending aorta

Variations in branching patterns of the descending thoracic aorta are common. The origins of the bronchial arteries, particularly those of the right side which are known to vary considerably in their number, usually arise from the third posterior intercostal artery. The oesophageal arteries anastomose with one another, but the formation of the anastomoses varies such that the number of oesophageal arteries ranges from 3-5.

Presence of variations in the origins of the posterior intercostal arteries is not well documented in the literature. However, in a study by Khan & Haust (1979) on 79 mortuary samples from children ranging from 1 day to 15 years, the distribution, size and numbers of the posterior intercostal arteries varied widely.

With respect to the abdominal aorta, variations in the branching pattern include aberrant renal and gonadal (particularly testicular) arteries as well as a common coeliacomesenteric artery.

Presence of a common coeliacomesenteric artery reportedly ranges from 2-5% (Rio Branco da Silva, 1912; Lippert & Pabst, 1985; Vandamme & Bonte, 1990; Douard et al., 2006), while the frequency of multiple renal arteries has been recorded in as many as 30% of individuals (Williams et al., 1995). The majority of supernumery renal arteries occur on the

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left side, although Janschek et al. (2004) observed multiple right-sided renal arteries in 20.2% of individuals and 19% on the left side of the body.

Variation in the origin of gonadal arteries is reported to be present in 8-15% of cadaver populations (Ciçekcibaşi et al., 2002; Pai et al., 2008; Terayama et al., 2008). In these cases, gonadal arteries arise more superiorly from the aorta, or from the renal or accessory renal arteries.

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2.4 Histological variation of the arterial wall

2.4.1 Cardiovascular disease

Pathological changes in the layers of the arterial wall can be direct causes of or can contribute to the development of cardiovascular disease (Burke et al., 1995; Griffin et al., 2009). The most well documented cardiovascular disease affecting the arterial wall is atherosclerosis.

2.4.1.1 Atherosclerosclerotic features of the vessel wall

Atherosclerosis is one of the most common forms of cardiovascular disease and is characterised by lesions in the tunica intima that protrude into the lumen of the vessel which can weaken the underlying tunica media (Kumar et al., 2005). These lesions are known as atheromas or atheromatous or fibro fatty plaques. The American Heart Association has classified atherosclerotic plaques into 6 Types, ranging from Type 1 (fatty dots or isolated macrophage foam cells) to Type 6 (complicated) lesions (Stary, 1994).
Clinical outcomes of atherosclerotic lesions include occlusion leading to stenosis, haemorrhage, thrombosis and formation of aneurysms.

Atherosclerosis was once thought to be a degenerative disease associated with aging, but research has shown that atherosclerosis is neither degenerative nor inevitable, and it is now thought to be an inflammatory condition (Virmani et al., 1991; Kumar et al., 2005).

The process of atherogenesis has been widely debated and a number of theories and models have been proposed. The most recent theories seem to include aspects from a number of models of atherogenesis, such as the response to injury hypothesis which proposes that atherosclerosis occurs as an inflammatory response to endothelial injury of the arterial wall (Virmani et al., 1991; Kumar et al., 2005). Endothelial damage can be caused by a number of factors including hypertension, hyperlipidaemia, smoking, haemodynamic forces and immune reactions. Such endothelial damage or injury causes endothelial dysfunction and increases the permeability of the endothelium, allowing monocyte adhesion and emigration into the tunica intima of the vessel. Once in the tunica intima, activated monocytes become macrophages and engulf oxidised low-density lipoproteins, which diffuse from the blood, to become foam cells. At the same time smooth muscle cells migrate to the tunica intima from the tunica media where they proliferate and deposit extracellular material. The increase of

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extracellular material leads to intimal thickening and in advanced stages of atherosclerosis this increase in extracellular material together with collagen and lipids (mostly in the form of cholesterol), forms the atherosclerotic plaques (Stary et al, 1994; Lusis, 2000; Fryan & Stanner, 2005; Kumar et al., 2005).

2.4.1.2 Risk factors for cardiovascular disease and arterial wall changes

Pathological changes to the arterial wall in cardiovascular disease are not only caused by disease processes but can also be induced or accelerated by risk factors such as smoking, obesity, diabetes, age, sex, genetics, ethnicity and HIV infection (see discussion below).

In South Africa it is estimated that the number of individuals who smoke is approximately 8 million, with a higher number of male than female smokers (van Rooyen et al, 2002), and a higher proportion of Coloured than African or White individuals (Yach et al., 1992). Long-term cigarette smoking is associated with impaired endothelium -dependent vessel vasodilatation and is known to accelerate the pathogenesis of CVD (Zeiher et al., 1995).

Obesity is a major problem worldwide and it is estimated that 1.4 billion people are overweight, while 500 million are obese (WHO, 2012c). In South Africa it is reported that 56% of women are obese, whilst men are significantly different with an average body mass index (BMI) that falls within the normal range (Puoane et al., 2002).
Increased BMI has been shown to cause an increase in cardiovascular risk and is therefore a major risk factor in the development of CVD (Li et al., 2006).

Type 1 and 2 diabetes mellitus is also known to accelerate CVD and it has been shown that the incidence of CVD in people with diabetes is 2-4 times greater than in non-diabetics (Haffner, 1998). Gao et al. (2007) has shown that insulin resistance together with obesity can also lead to endothelial dysfunction.

Currier et al. (2003) indicated that individuals with HIV are more likely to develop CVD than individuals who are HIV-negative. HIV has been reported in higher proportion in vessel walls of atherosclerotic plaques (Bobryshev, 2000). The histological changes due to HIV infection are discussed later on. Mechanisms by which HIV affects the vascular system are still unknown, however it is suggested that an increase in cytokines leads to a dysfunctional

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endothelium (Currier et al., 2003). Anomalies caused by highly active antiretroviral treatment, such as irregular glucose metabolism and lipodystrophy, have also been linked to increased risk for developing atherosclerosis and CVD (Metroka, 2007). With HIV infection rates increasing globally, but particularly in Sub-Saharan Africa (WHO, 2006; WHO, 2012b), this is a major risk factor for CVD in the South African population.

2.4.2 Documented histological variation

Van Kets et al. (2011) described two categories of potentially pathological histological changes in a South African population. The first category comprised of structural changes in the vessel wall (Figure 2.2) and the second comprised the presence of abnormal cells or cellular incursions (Figures 2.3 and 2.4). This section looks to the literature in trying to explain these changes.

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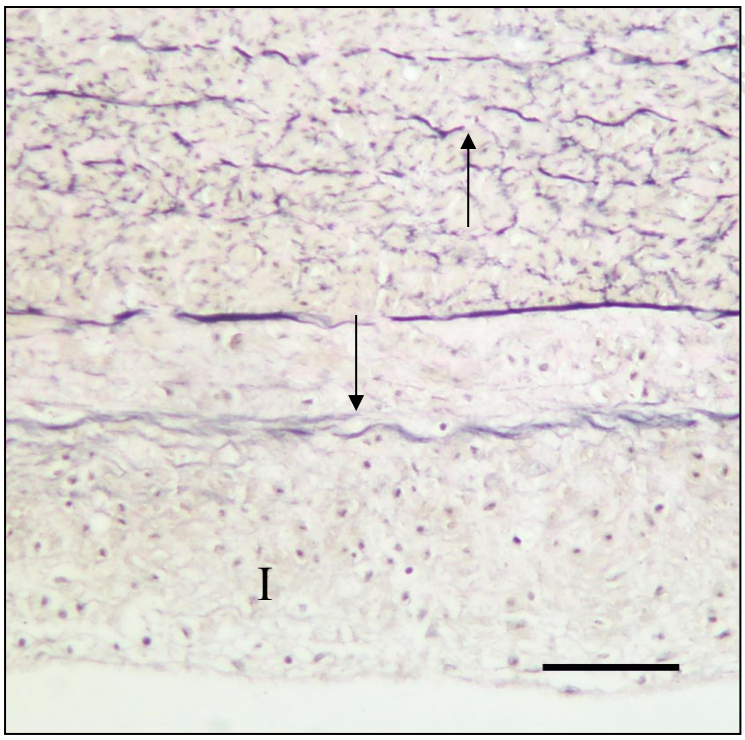

Figure 2.2: Fragmentation and 'splitting' of elastin fibres, with thickening of the tunica intima in a 26 year old African male (EVG stain)
Arrows: 'splitting' of elastin fibres, I: Tunica intima, Scale bar: 100µm
(Micrograph used with permission from Van Kets)

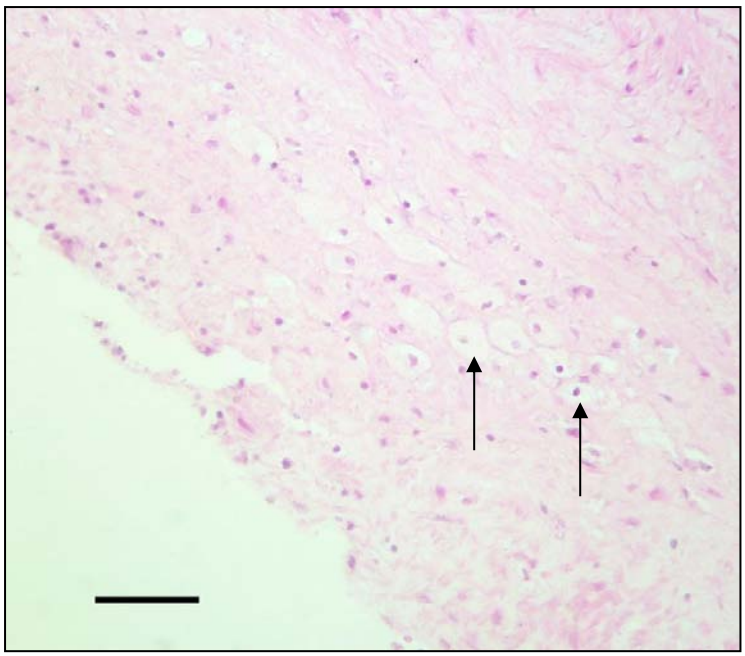

Figure 2.3: Foamy macrophages in the tunica intima of a 37 year old Coloured male (H&E stain)

Arrows: Foamy macrophages, Scale bar: 100µm

(Micrograph used with permission from Van Kets)

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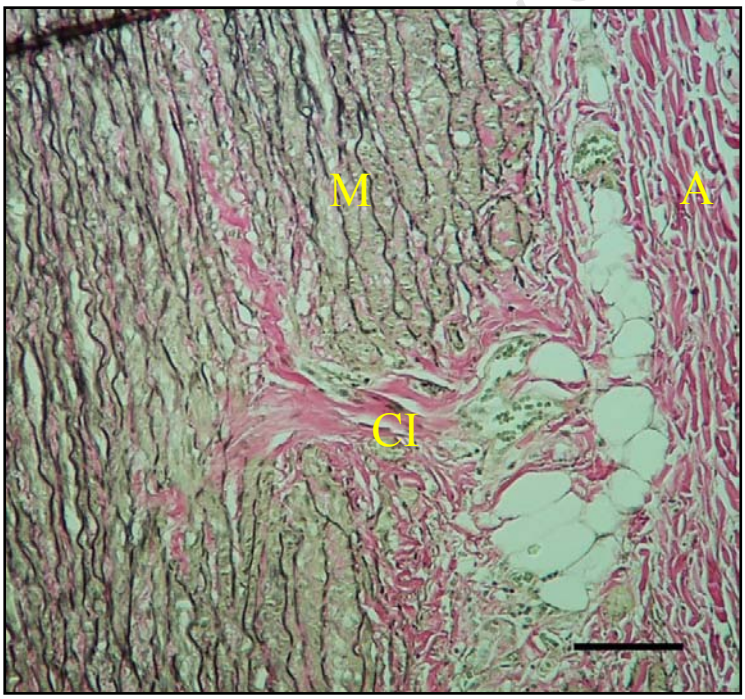

Figure 2.4: A collagen 'incursion' extending from the tunica adventitia into the tunica media in an 18 year old Coloured male (EVG stain)

A: Tunica adventitia, CI: Collagen incursion, M: Tunica media, Scale bar: 100µm

(Micrograph used with permission from Van Kets)

2.4.2.1 Atherosclerotic changes

Histological changes suggestive of atherosclerosis were found to be common amongst a South African mortuary sample of 150 individuals (Van Kets, 2007; Van Kets et al., 2011). According to Stary et al. (1992) intimal thickening is a key histological feature of atherosclerosis. However, intimal thickening on its own may not necessarily be indicative of pathology. Intimal thickening is also associated with vessels such as the aorta that are subject to haemodynamic forces such as reduced shear stress (Carallo et al., 1999), as discussed in the section below on haemodynamics. Other atherosclerotic changes identified by Van Kets et al. (2011) include presence of foam cells, increased numbers of lymphocytes, lipid pools and cholesterol clefts (Stary et al., 1992).

2.4.2.2 Non-atherosclerotic diseases

Non-atherosclerotic diseases that affect the histological structure of the arterial wall include intimomedial mucoid degeneration, cystic medial degeneration, Marfan syndrome, Ehlers-Danlos syndrome, aortitis, and congenital heart disorders (Virmani et al., 1991; Gawthrop et al., 2007). These non-atherosclerotic diseases are discussed in the sections below.

2.4.2.3 Elastin fragmentation

Elastin fragmentation is characterised by the disruption of the elastin lamellae and is a common histological characteristic of the aging human aorta (Schlatmann & Becker, 1977; Cattell et al., 1996). It may also be present in annuloaortic ectasia, Marfan syndrome, HIV-positive status and congenital heart disorders such as tetralogy of Fallot (Halme et al., 1985; Hirata et al., 1991; Chetty et al., 2000; Tipping et al., 2006).

Elastin is found in the tunica media of elastic and muscular arteries and is produced from proelastin in young aortic smooth muscle cells (Ross, 1971). The function of elastin is to resist the forces and tensile stresses generated by the pulsatile action of the blood.

Elastin fragmentation or disruption was a frequent histological characteristic described by Van Kets et al. (2011). Schlatmann & Becker (1977) noticed that elastin fragmentation

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varied widely in the aging aorta. These authors developed a grading method to quantify the extent of elastin fragmentation. This method was based on the number of foci of elastin fragmentation seen within a specific microscopic field, and identified 3 categories of severity of elastin fragmentation. Elastin fragmentation was not seen in the aorta of individuals aged six years or less. Schlatmann & Becker (1977) concluded that haemodynamic forces were responsible for initiating the degeneration of elastic tissue in the aging aorta. With regard to aging and elastin fragmentation, it is furthermore noted that the amount of elastin in the aorta decreases with age, but that the concentration or proportion of elastin in the aorta increases (Cattell et al., 1996). This could suggest that other components of the aortic wall are lost more rapidly with aging than elastic tissue.

As mentioned earlier elastin fragmentation is not only associated with aging, but may also be a consequence of pathology. Annuloaortic ectasia is a term used to describe a dilation of the ascending aorta and is often, but not necessarily associated with Marfan syndrome. Histological characteristics of annuloaortic ectasia include the presence of varying amounts of fibrosis and elastin fragmentation (Halme et al., 1985), which are similar to the histology of vessels in Marfan syndrome. Marfan syndrome is an autosomal dominant genetic disorder that affects the connective tissue and is characterised by abnormalities of the eyes, skeleton, and cardiovascular system (Virmani et al., 1991). Patients with Marfan syndrome have abnormal properties of the elastin in their bodies, particularly the ascending and abdominal segments of the aorta (Hirata et al., 1991). The extent of elastin fragmentation within the tunica media of patients with Marfan syndrome varies. Severe cases showing fragmentation and complete loss of elastic lamellae, whilst milder cases may only present with mild focal elastic tissue loss (Halme et al., 1985).

Elastin fragmentation of arterial vessel walls has also been associated with HIV infection (Chetty et al., 2000; Tipping et al., 2006). Tipping et al. (2006) investigated intracranial aneurysms in HIV positive adult women and found degenerated elastic laminae, mucoid degeneration, and atrophy of the tunica media causing consequent ectasia of the intracranial vessels. It was suggested that altered levels of circulatory cytokines and growth factors are responsible for the observed pathologies. Chetty et al. (2000) made similar observations in sixteen HIV-positive patients with either large vessel aneurysm or large vessel occlusive disease. Histological findings included loss and fragmentation of the elastic tissue in the tunica media with fragmentation of the internal elastic lamina of the tunica intima. These two

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studies confirm that HIV has a definite impact not only on the elastic tissue, but other constituents of the vessel wall too.

2.4.2.4 Fibrosis

Fibrosis is defined as an increase in collagen fibres and it is generally accepted that the aorta exhibits increased levels of collagen with age (Schlatmann & Becker, 1977). However, fibrosis is also present in the aorta of patients with hypertension. Szmigielski et al. (2006) suggested that the collagenase-anticollagenase system is abnormal in hypertensive patients and may contribute to increased collagen in the tunica media. Haemodynamics factors (Carallo et al., 1999) and genetic influences (Kingwell et al., 2001) may also affect collagen increases in the aortic tunica media. Fibrosis in the arterial wall is therefore either described in relation to pathology, such as hypertension or is suggestive of physiological factors such as aging, genetics and haemodynamics.

2.4.2.5 Cystic medial necrosis

Cystic medial necrosis was first described by Erdheim in 1930, but has more recently been defined as the pooling of proteoglycans and the appearance of cyst-like structures in the tunica media with the term medionecrosis as the loss of cell nuclei in the media (Schlatmann & Becker, 1977). The condition can also be described as the degradation of collagen, elastin and smooth muscle cells and may be present to a certain degree in normal aging aortae. The most severe cases of cystic medial necrosis, as with elastin fragmentation, are seen in Marfan syndrome. Tetralogy of Fallot and coarctation of the aorta are also associated with cystic medial necrosis.

Tetralogy of Fallot is a congenital heart disorder which involves four abnormalities of the heart (pulmonary stenosis, an overriding aorta, ventricular septal defect and right ventricular hypertrophy). Tan et al. (2005) investigated intrinsic histological abnormalities of the aortic root and ascending aorta in tetralogy of Fallot and concluded that there were marked histological changes in the aortic wall from infancy. These changes included cystic medial necrosis, medionecrosis, fibrosis, and elastic fragmentation with elastic lamellae disruption.

Aortic coarctation is the term used to describe narrowing of the aorta in the area of the ductus arteriosus and three subtypes are described according to the precise location of the narrowing.

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Isner et al. (1987) examined the light microscopic features of coarctation segments of aorta obtained from either surgery or autopsy. Results indicated that in the majority of coarctation specimens the extent of cystic medial necrosis was severe. This represents a consistent histological finding in coarctation of the aorta.

2.4.2.6 Acid Mucopolysaccharides

Mucopolysaccharides are a class of polysaccharide molecules, also known as glycosaminoglycans, composed of amino-sugars chemically linked into repeating units that give rise to a linear unbranched polymeric compound and are a constituent of connective tissue (The Columbia Electronic Encyclopaedia, 2003). Acid mucopolysaccharides are found in normal aortae and are associated with collagen in juveniles and adults, and with the absence of collagen in fetuses and infants (Zugibe, 1962).

Large amounts of acid mucopolysaccharides are associated with intimomedial mucoid degeneration. Intimomedial mucoid degeneration has been implicated in aneurysms in the Black South African population (Decker et al., 1977; Abdool-Carrim et al., 1996). Intimomedial mucoid degeneration shows similar histological characteristics to cystic medial necrosis and includes diffuse elastic tissue degeneration with large quantities of acid mucopolysaccharide deposits within the tunica media and intima (Abdool-Carrim et al., 1996). Cystic medial necrosis differs as it only affects the tunica media of blood vessels, whereas intimomedial mucoid degeneration includes both the tunicae intima and media.

Diet may also have a role in the presence of acid mucopolysaccharides in the vessel wall. Sandhyamani (1992) investigated vasculopathic and cardiomyopathic changes induced by a high-carbohydrate low-protein based diet in monkeys. The author of this study concluded that the results established an etiologic role for diet, especially protein deficiency, in the induction of vascular change.

2.4.3. Haemodynamic forces

Haemodynamics refers to the study of movement of blood and of the forces associated with it. Haemodynamic forces have been correlated with the pathological changes seen in atherosclerosis (Davies, 1997; Kumar et al., 2005).

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2.4.3.1 Atherosclerotic prone areas

Atherosclerotic lesions tend to develop in regions where there is a disturbance of the unidirectional laminar blood flow (Zarins et al., 1983; Asakuru & Karino, 1990; Glagov et al., 1992; Giddens et al., 1993). This typically occurs near branching points of vessels, bifurcations, or regions of arterial narrowing and curvature in vessels. Certain blood vessels and areas of specific arteries seem to be more susceptible than others, for example at the carotid bifurcation, the coronary arteries, the abdominal aorta and the iliofemoral arteries, whilst others seemed to be spared despite their complex pattern of blood flow (Davies, 1997). Such arteries include the internal mammary and renal arteries.

Several studies have investigated the role of haemodynamic forces and their effects on the arterial wall, particularly in respect of atherogenesis. Favoured arteries for these studies seem to be the carotid and coronary arteries due to their bifurcations and curved vessel structure. The role of haemodynamic factors in atherogenesis has been investigated using flow pattern visualisation and it has been suggested that haemodynamic mechanisms for lesion formation include flow velocity and shear stress, decreased wall shear and turbulence (Zarins et al., 1983; Giddens et al., 1993). Shear stress refers to the frictional force that acts tangentially to the endothelial surface (Carallo et al., 1999).

Zarins et al. (1983) investigated the localisation of atherosclerotic plaques in the carotid bifurcation with regards to flow velocity profiles and wall shear stress. This study found that moderate and uniform intimal thickening of the vessel was correlated with flow profiles that were axisymmetric, where flow streams were unidirectional and axially aligned, and where there was a raised shear stress. Conversely, there was three to five times thicker eccentric intimal thickening in the vessels where there were complex flow patterns, vortex formation, reduced velocity, retrograde flow, and reduced shear stress.

Similarly, Asakura & Karino (1990) investigated flow patterns and spatial distribution of atherosclerotic plaques in the coronary arteries. It was found that the outer wall at the sites of bifurcations and the inner wall of curved segments of coronary arteries were more prone to atherosclerotic plaque formation. They concluded that reduced fluid velocity and resultant low shear stress was directly related to the localisation of the atherosclerotic plaques.

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These studies confirm that reduced fluid velocity, reduced shear stress and flow that are not unidirectional and axially aligned (i.e. turbulent) are haemodynamic factors that promote atherogenesis.

By contrast, Aars & Solberg (1971), who investigated the effect of turbulence on development of aortic atherosclerosis in rabbits on high cholesterol diets, found that turbulence and dilation of the aortic arch did not influence the localisation or extent of plaque formation.

2.4.3.2 Haemodynamics and endothelial function

A healthy endothelium has many functions and regulates several different processes, which include coagulation, growth of underlying smooth muscle cells, leukocyte adhesion to and transmigration into the vessel wall, and lipoprotein uptake and metabolism. It is well established that transduction of both biochemical and biomechanical stimuli determine the physiology or pathology of the cardiovascular system (Resnick et al., 2003).

Shear stress and cellular biology are thus intertwined and mechanical forces such as shear stress are important modulators of cell function, particularly in the cardiovascular system. A dysfunctional endothelium results from reduced shear stress and flow reversal. This causes increased uptake of lipoproteins, transmigration of leukocytes and the appearance of leukocyte adhesion molecules on the luminal surface of endothelial cells (Traub & Berk, 1998; Cunningham & Grothib, 2005). A dysfunctional endothelium also leads to proliferation of monocytes/macrophages and smooth muscle cells due to secretions of chemotactic factors and growth factors (Cunningham & Gotlieb, 2005). Smooth muscle cells are thus able to produce connective tissue matrix, which is comprised of elastic fibres, proteins, collagen and proteoglycans leading to the development of cardiovascular disease, particularly atherosclerosis.

Gene expression in endothelial cells exposed to blood flow is discussed in a review by Resnick et al., (2003). It is believed that there are forty genes that are regulated by low shear stress and their expression increases or decreases in response to blood flow. Low shear stress is capable of changing the transcriptional activity of endothelial cells. Some genes are found in vivo in areas of atherosclerosis or inflammation, whilst other genes are anti-atherogenic

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and function as inhibitors of proliferation, adhesion, thrombogenesis and inflammation. Gene therapy in this regard is therefore an area of much interest.

2.5 Summary and conclusion of the literature review

2.5.1 Variation in the branching pattern of the aorta

From the literature it is clear that gross anatomical variation, with a range of possible combinations of branching patterns along the aorta, occurs at varying frequencies. However, the anatomy and variations in the branching patterns of the aorta among South African populations are poorly represented in this literature. The present study aims to address this issue and contribute to the knowledge of variation in the branching patterns of the human aorta.

2.5.2 Histological variation of the arterial wall

Histological changes in the aortic wall may be due to a combination of pathology and physiological processes. Whether physiological processes such as haemodynamical forces cause these changes early on in the fetus, allowing risk factors and pathological processes to compound the problem and to assist in the further development of these changes is yet to be determined. This study intends to address the question of determining which particular histological features can be associated with haemodynamics by looking at particular regions of the human aorta known to be associated with haemodynamic forces. This may allow for the interpretation of microscopic changes reported in the South African population.

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Chapter 3: Materials and methodology

3.1 Materials

The materials used in this research were collected from two sources, a cadaver sample from the Department of Human Biology, University of Cape Town (UCT), and a mortuary sample from the Salt River Mortuary, Cape Town. With respect to the UCT cadaver sample, the aorta and its branches were dissected in situ and were used to document the variation of branching patterns of the aorta. The mortuary samples were used primarily to investigate the histological structure of both the aorta and any of its branches that were removed with the main vessel sample.

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3.1.1 Ethical approval

Ethical approval for this research was granted by the Faculty of Health Sciences Human Research Ethics Committee of the University of Cape Town (REC REF: 227/2005).

3.1.2 Cadaver sample

A total of 71 cadavers were dissected during 2008 and 2009. Of the 71cadavers, 42 were White individuals (25 males, 17 females), 15 were Black individuals (14 males, 1 female), and 14 were Coloured individuals (13 males, 1 female). The age of the cadavers ranged from 20-102 years, with an average age of 65 years. Basic demographic data for all cadavers used in the Department of Human Biology, UCT, are routinely entered and stored in a hand-written ledger in the Department. Data include the sex, age and race of the individuals. These data for the 71 cadavers in this study were entered into a Microsoft Office Excel 2007 spreadsheet and then transferred into Statistica 8 for statistical analysis.
The cadavers, which were used for the MBChB dissection block of the programme, had already been partially dissected by students prior to this study, so most of the major vessels had already been exposed.

3.1.3 Mortuary sample

Portions of aortae from a total of 228 randomly selected individuals were collected for histological examination from Salt River Mortuary in Cape Town, during procedural

autopsies performed by pathologists from the Department of Clinical Laboratory Sciences, University of Cape Town. Sections from as many regions as possible (the ascending, arch, descending and abdominal aorta as far as the bifurcation into paired iliac arteries) were collected. Unfortunately it was not possible to examine the aortae in situ or as complete vessels and therefore there was no opportunity to determine aortic branching patterns in the mortuary sample.

Where possible, demographic data (age, sex, and race) for each individual were collected from the records of the South African Police Services (see Appendix 1).

Of the 228 individuals sampled, 126 were Black individuals (111 males, 15 females), 68 were Coloured individuals (53 male, 15 female), and 34 were White individuals (23 male, 11 female). The age of these individuals ranged from 10 to 88 years, with an average age of 37 years.

As part of routine forensic examination the pathologists bisect the aorta longitudinally and examine the macroscopic appearance of the luminal surface from the proximal ascending aorta to the point of bifurcation into the common iliac arteries. The gross appearance of the vessel is recorded and categorised as normal, fatty streaks, plaques, ulcerations, calcifications, haemorrhage, thrombus or aneurismal (see Appendix 1 for a copy of the data recording sheet). At collection, samples were placed in plastic jars containing 10% neutral buffered formalin and labelled according to the serial number allocated to each post-mortem case by the South African Police Services. The samples were then taken to the Department of Human Biology, UCT, and stored for no less than one month to ensure proper fixation.

Prior to tissue processing, the aorta samples were examined and details of the specific sections collected were recorded. Specimens with macroscopic evidence of atherosclerosis or those which excluded one or more of the regions of the aorta were not included. There were 25 complete aortae samples with no evidence of macroscopic pathology and thus 25 samples were used for the histological examination.

During assessment of the luminal surface of the mortuary samples collected, the presence of an aortic ridge at the junction of the aortic arch and descending aorta was noted. Of the total of 228 samples collected, 150 included the junction of the aortic arch and descending aorta

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where the ridge was noted. These 150 samples were used to document the presence of this aortic ridge and investigate its histological structure. One of the samples was disregarded due to decomposition and therefore 149 samples were finally used. The cadaver samples were not examined for the ridge as the cadaver population was thought to be too skewed. It was thought that the mortuary population was a better representation of the living population in South Africa and that examining for the ridge in these samples would produce more accurate results in terms of population.

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3.2 Methodology

3.2.1 Variation in the branching pattern of the aorta

In the cadavers, the aorta and its branches were dissected in situ from the level of the ascending aorta to a point just distal to the bifurcation into the right and left common iliac arteries. The dissection process involved careful removal of any tissues, particularly fascia, from around the blood vessels. Once the aorta and its branches were fully exposed, the identification of the branching vessels and their relative positions to key anatomical landmarks in situ were documented (see Appendix 2 for an example of the data sheet used to capture this information). Evidence of variations or anomalies in the vessels was recorded. All data were initially recorded by hand on data sheets and subsequently transferred into Microsoft Office Excel 2007 for analysis. The dissected vessels and their branches were also photographed using a Sony Cyber-shot 7.2 mega pixel camera.

Following dissection, the complete aorta and the proximal sections of each of the branches, where possible, were removed from each of the cadavers. A metal tag with the cadaver identification number was sutured to each aorta, which was then stored in a large plastic container containing neutral buffered formalin.

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3.2.2 Histological variation of the aorta and branching points

Histological variation was documented using the mortuary sample. From each of the samples collected, four main regions were selected for histological examination. Tissue samples taken from each of these regions were processed, sectioned and stained using standard histological methods. The sites chosen and the methods used for histological examination are outlined below.

3.2.2.1 Selected histological sites

The four regions and the sites from which tissues were removed for histological examination are shown in Figures 3.1-3.4. In all, a total of seven sections were taken for analysis from each of the 25 samples. The 4 regions include:

I. At the level of the ascending aorta, at a point just proximal to the aortic arch and parallel to the origins of the 3 main branches of the aortic arch (brachiocephalic trunk, left common carotid and left subclavian arteries).

II. Between the 6^{th} and 7^{th} paired posterior intercostal arteries in the descending thoracic aorta. Sections from the dorsal and ventral walls of the vessel were taken at this level (Figure 3.2).

III. At the level of the right renal artery. The origin of the right renal artery and sections of the superior and inferior portions of the descending abdominal aorta on either side of the renal artery were dissected out. A coronal section was then taken at the point where the right renal artery branches off the aorta. Two sites were available; superior and inferior renal (Figure 3.3).

IV. At the bifurcation of the abdominal aorta, into left and right common iliac arteries. The point of bifurcation was dissected out, and a coronal section was taken at the point where the aorta bifurcates into the two common iliac arteries. Two sites were available at this region: the tip of the bifurcation and a site just distal to the tip (Figure 3.4).

Note that these regions were decided upon by me and my supervisors to incorporate areas of branching and non-branching sites along the entire length of the aorta.

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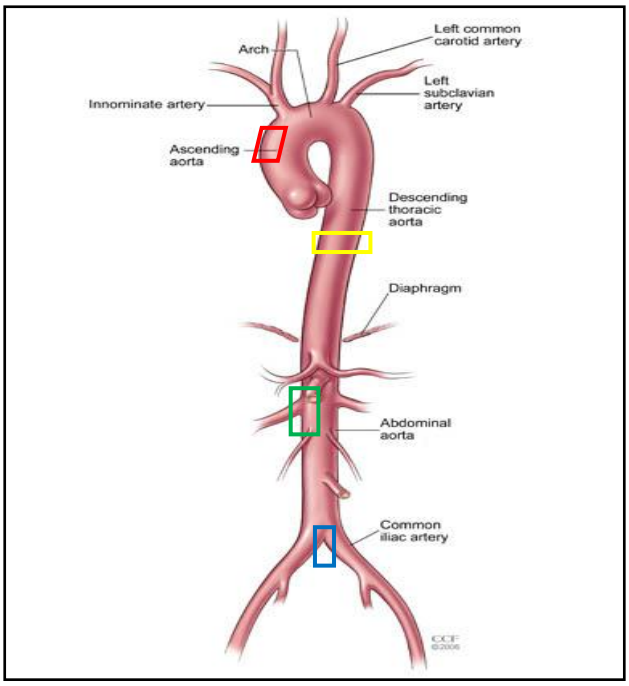

Figure 3.1: Diagram of the aorta illustrating the 4 sites chosen for histological examination Red: ascending site, Yellow: intercostal site, Green: renal site, Blue: bifurcation site

(Image adapted from https://www.clevelandclinic.org/heartcenter/images/guide/disease/marfan/aortaLG.jpg)

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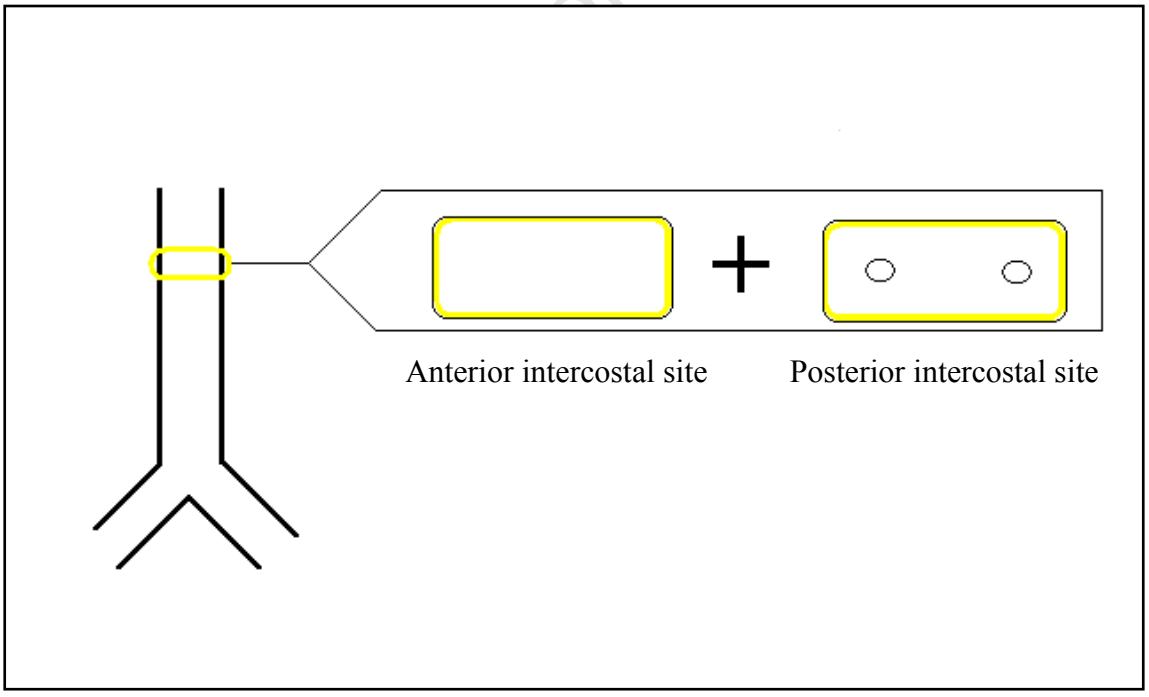

Figure 3.2: Diagram of the descending (thoracic) aorta illustrating the position of the two sections taken at between the 6th and 7th paired posterior intercostal arteries

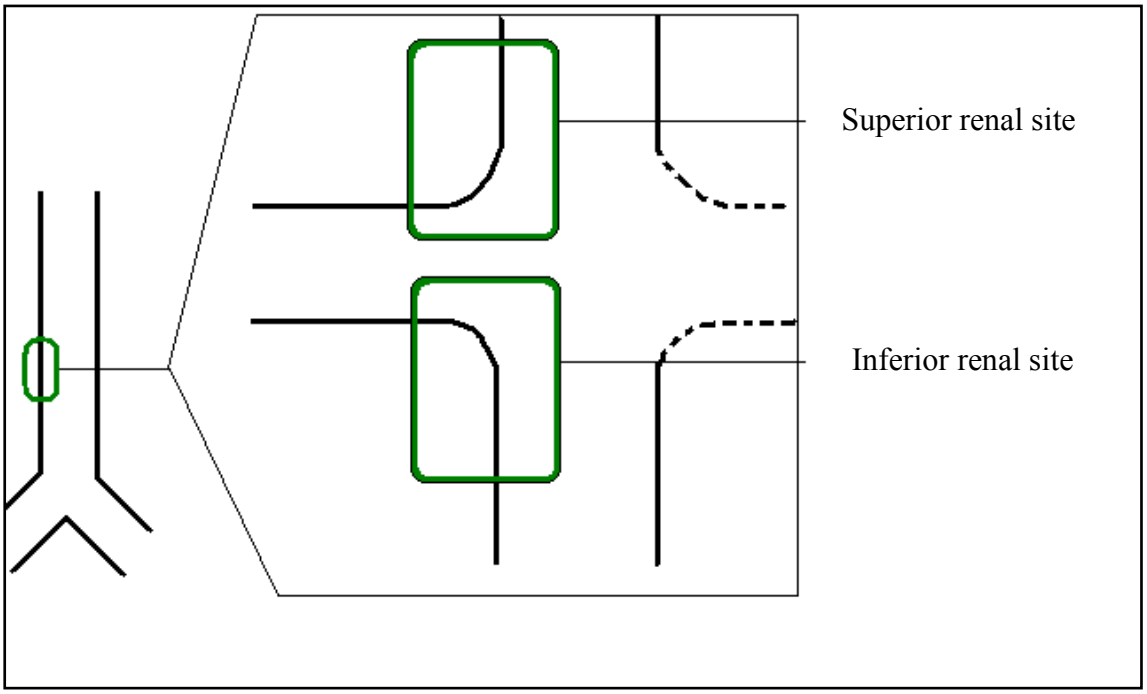

Figure 3.3: Diagram of the abdominal aorta illustrating the position of the two sections taken at the level of the right renal artery

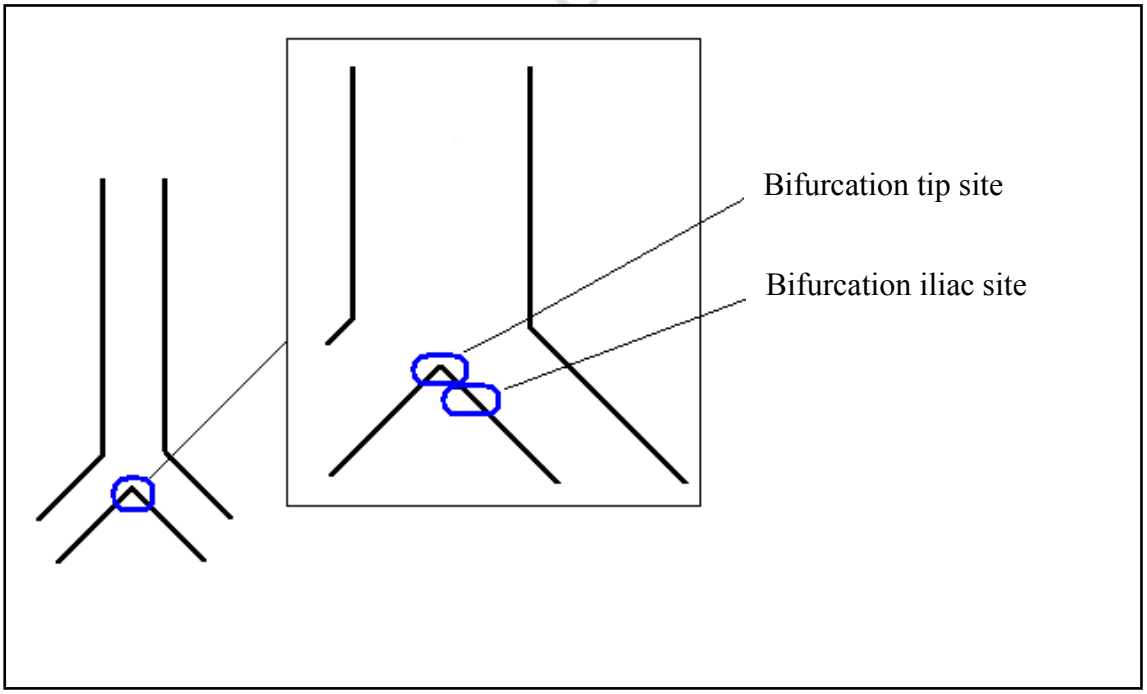

Figure 3.4: Diagram of the abdominal aorta illustrating the position of the two sections taken from the bifurcation

3.2.2.2 Histological processing

Tissue samples, which were approximately 1.5 x 0.5 cm in size, were placed in small metal mesh containers for tissue processing. Tissues were dehydrated by immersion in increasing concentrations of alcohol, followed by immersion in xylol and finally melted paraffin wax. The SE 400 automated tissue processing machine (Shandon Elliot) was used for this procedure. Below is a summary of the tissue processing procedure.

Beaker	Solution	Time in Solution	Setting on SE 400 processing machine	Actual Time
Beaker 1	10% Buffered Formalin	1 Hour	(0-1)	12-1 pm
Beaker 2	70% Alcohol	1 Hour	(1-2)	1-2 pm
Beaker 3	70% Alcohol	1 Hour	(3-4)	2-3 pm
Beaker 4	90% Alcohol	1 Hour	(2-3)	3-4 pm
Beaker 5	90% Alcohol	2 Hours	(4-6)	4-6 pm
Beaker 6	Absolute Alcohol	2 Hours	(6-8)	6-8 pm
Beaker 7	Absolute Alcohol	2 Hours	(8-10)	8-10 pm
Beaker 8	Absolute Alcohol	2 Hours	(10-12)	10-12 pm
Beaker 9	Xylol	2 Hours	(12-14)	12 pm-2 am
Beaker 10	Xylol	2 Hours	(14-16)	2-4 am
Beaker 11	Wax	2 Hours	(16-18)	4-6 am
Beaker 12	Wax	4 Hours	(18-->)	6-10 am

Table 3.1: Tissue Processing Schedule

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Once processed, the tissues were embedded using a Kunz WD-4 embedding machine. Moulds containing the tissues and wax were then placed on an Axel Johnson Lab CPL-4 cooling plate to solidify the wax blocks.

3.2.2.3 Histological sectioning

Wax blocks containing embedded samples were cut using a Reichert-Jung Autocut 2040 microtome at a thickness of 5µm. Sections were floated out on 30% alcohol followed by a water bath and then lifted onto glass slides. This process of floating the sections helps to flatten the section and prevents wrinkling. The glass slides were then left overnight to dry in an incubator at 62° C.

Hydration was performed on the sections that were to be stained using standard histological stains. Before the hydration process, the wax needed to be removed from the tissue. This is achieved using three baths of xylol after which hydration is performed using decreasing concentrations of alcohol, 3 baths of absolute alcohol, 2 baths of 90% alcohol, and 1 bath of 70% alcohol. The tissue is then rinsed in running tap water to complete the hydration process.

3.2.2.4 Histological staining

Hydrated sections were stained with Haematoxylin and Eosin (H&E), Elastin von Gieson's (EVG) stain, and Alcian Blue pH2.5 - Periodic Schiff reactions (AB-PAS) (see Appendices 3 and 4). The H&E stain was used to determine the general appearance and morphology of the vessel walls. The EVG stain was used to visualize the elastic and collagen tissue, as well as smooth muscle cells, within the arterial wall. Elastic tissue stains black, collagen stains a red to pink colour, and smooth muscle cells stain a yellow to green colour. The AB-PAS was used to distinguish the presence of acid and neutral mucopolysaccharides in the arterial wall. Acid mucopolysaccharides stain blue, whilst neutral mucopolysaccharides stain pink.

3.2.2.5 Waste disposal

All chemicals were disposed of as per the guidelines maintained by the histology laboratory in the Department of Human Biology, UCT.

3.2.2.6 Examination of histological features

The structure of the arterial wall was examined by light microscopy. The general appearance of the vessel walls was recorded, as were any structural modifications not typically found in a 'normal' vessel.

These structural alterations include thickening of the tunica intima, elastin degradation, increased collagen in the vessel wall, or atherosclerotic changes such as cholesterol clefts and plaques. Presence of cells not typically found in a vessel, for example foamy macrophages or large numbers of lymphocytes was also documented.

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Images of stained sections were digitalized using an Axiocam High Resolution colour camera on a Zeiss Axioskop Mot upright microscope. These images were delivered to Axiovision 4.7 software and stored as Tagged Image File Format or TIFF files. Once captured, the images were transferred to *Image J* software for analysis and quantification of histological features.

Histological features of each sample site were recorded in Microsoft Office Excel 2007. The data from the sample sites of each individual aorta was summarised in order to compare the different sample sites within the whole aortic sample. Data were transferred to Statistica 8 for statistical analysis.

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Acid mucopolysaccharides

Acid mucopolysaccharides were either present or absent from aortic sections sampled. Presence of acid mucopolysaccharides was determined by a positive blue stain from the AB-PAS stain. If a section did not stain blue, acid mucopolysaccharides were absent from the sampled section. Images of the tissue samples were also stored in *Image J*, and presence of acid mucopolysaccharides was recorded in the Microsoft Office Excel 2007 sheet containing the other histological features for each sample site of each aorta.

Graphs and tables were generated using Microsoft Office Excel 2007.

Vessel wall measurements

Measurements of the tunica intima and tunica media thickness of each sample were measured by *Image J.* At each site, 3 individual measurements were taken to obtain an average thickness for each site. This was achieved by drawing three straight, equidistant lines from the intimal surface to the adventitia on the image of the vessel wall (Figure 3.5). The tunica intima thickness was measured from the luminal surface to the internal elastic lamina, whilst the tunica media thickness was measured from the internal elastic lamina to start of the tunica adventitia. The demarcating of the different tunica layers was clearest when viewing the samples stained with the EVG stain and thus the EVG images were used for the tunica measures.

A macro (an abbreviation for a set of commands) was created in *Image J* to ensure correct scales for each image taken at different magnifications and also included a setting to display a

250 by 250 micrometer grid on the image. This grid would enable the three measurements to be taken at equally spaced intervals.

The three tunica thickness measurements thus gave an average tunica thickness at each site. In order to obtain an average tunica intima thickness at each sample site for the whole sample, the 25 average tunica intima thicknesses at each site were then averaged.
For example; three measurements were taken at the ascending aorta site of one individual in order to obtain an average diameter for that particular ascending site. This was repeated for the other 24 ascending sites from the remaining 24 individuals. The average thickness for the ascending site was then calculated from the 25 average thicknesses of the ascending site from the 25 individuals.

The measure function under the Analyse tab was used to measure the tunica intima and tunica media along the drawn lines on the image. A summary of the measurements taken was created in *Image J* for each image and transferred into Microsoft Office Excel 2007. Graphs and tables were created in Microsoft Office Excel 2007, while statistical analysis was performed in Statistica 8.

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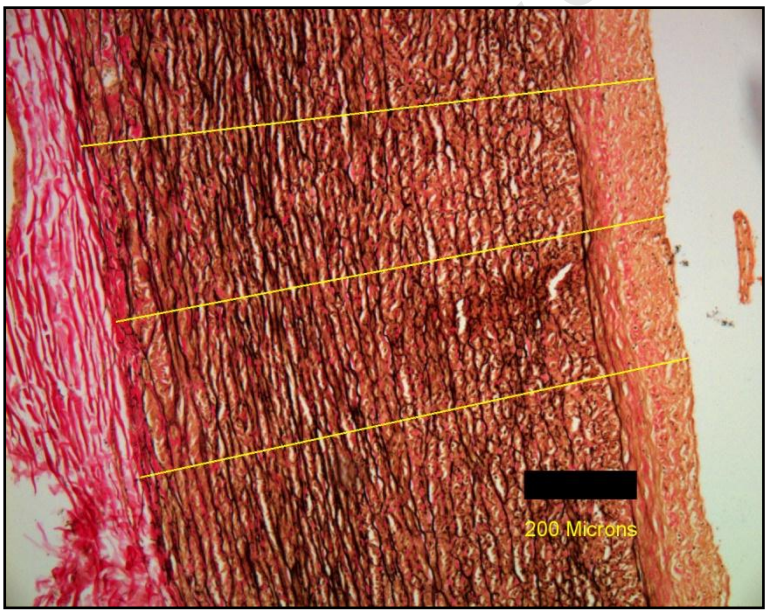

Figure 3.5: Micrograph demonstrating lines along which tunica thickness measurements were taken along (section taken from the superior renal site of a 26 year old Black male, EVG stain)

Intimomedial ratio

The intimomedial ratio was calculated as the (mean tunica intima value) / (mean tunica media value) for each site. This was calculated using Microsoft Office Excel 2007. In order to compare if there was tunica intimal thickening at different sites along the length of the aorta, the ratio rather than the raw tunica measurements were used. This was because the raw tunica intima and media measurements changed along the length of the aorta.

Elastin fragmentation

Schlatmann & Becker (1977) designed a grading system for establishing degrees of elastin fragmentation in order to quantify elastin fragmentation in the aging human aorta. According to these authors, elastin fragmentation is defined as focal fragmentation of elastin lamellae in the tunica media of the aorta. The grading system is based on the number of focal areas (or foci) of elastin fragmentation within a single microscopic field of view. The system proposes 3 grades (1, 2 & 3), to reflect increasing levels of elastin degradation according to the number and extent of the foci.

The system designed by Schlatmann & Becker (1977) was used in this research as is outlined below.

Grading system by Schlatmann & Becker (1977):

Grade 1: samples of aorta with fewer than 5 foci with elastin fragmentation in 1 microscopic field (200X). Note that each focus must comprise 2-4 neighbouring elastin lamellae. Interruption of one elastin fibre alone was not interpreted as fragmentation. The orientation of smooth muscle cells is preserved. See example of Grade 1 in Figure 3.6.

Grade 2: samples of aorta with more than 5 foci with elastin fragmentation in 1 microscopic field (200X). Each focus must comprise 2-4 neighbouring elastin lamellae and foci could be either confluent or scattered throughout the tunica media. The orientation of smooth muscle cells is preserved. See example of Grade 2 in Figure 3.7.

Grade 3: samples of aorta with foci showing elastin fragmentation in five or more elastin lamellae within 1 microscopic field (200X), irrespective of the number of foci present. See example of Grade 3 in Figure 3.8.

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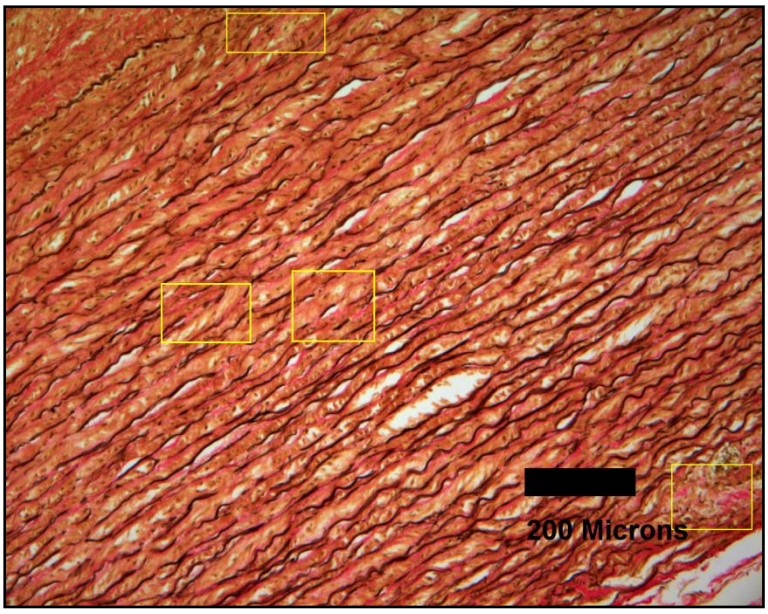

Figure 3.6: Grade 1 elastin fragmentation in a section taken from the anterior intercostal site of a 45 year old White male (EVG stain)

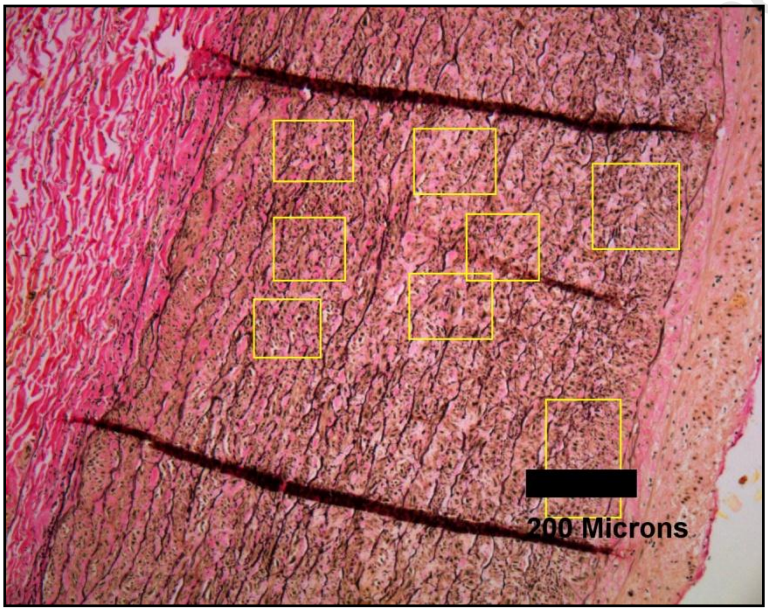

Figure 3.7: Grade 2 elastin fragmentation in a section taken from the superior renal site of a 30 year old Black male (EVG stain)

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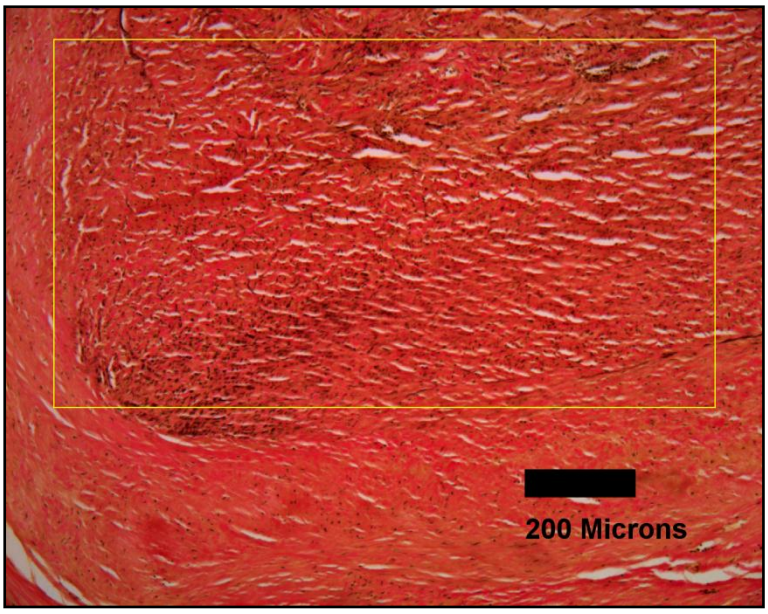

Figure 3.8: Grade 3 elastin fragmentation in a section taken from the superior renal site of a 55 year old Coloured male (EVG stain)

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3.2.2.7 Observer bias for quantification of histological features

Observer bias was assessed by repeated observations on slides stained for presence of acid mucopolysaccharides, thickness of the tunica intima and media and elastin fragmentation. This comprised two observation sets performed two months apart, on 5 randomly selected samples. The 5 randomized samples were chosen using a statistical program STATA (available online at www.stata.com). There was no statistically significant difference between the two measuring occasions ($t=2.20$, $p=0.16$) therefore it can be concluded no observer bias was noted during quantification of the histological features during this research.

3.2.3 Histological variation of the aortic ridge

The presence of an aortic ridge at the junction of the aortic arch and descending aorta was noted during assessment of the luminal surface of the mortuary samples collected. Of the 228 samples collected, 149 samples were used to document the presence of this aortic ridge and investigate its histological structure (150 included the junction of the aortic arch and descending aorta where the ridge was noted, but one of the samples was disregarded due to decomposition).

The presence of the aortic ridge was documented by hand in a table including the sample number. This data was then entered into a Microsoft Office Excel 2007 spreadsheet for further analysis.

The histological features of the ridge were investigated in 10 of the 149 aortic samples collected from the Salt River Mortuary. Two sections were taken from each aortic arch in the subsample of 10 randomly selected (using the statistical program STAT) individuals. The first section was taken from a normal macroscopic non-branching area where the aortic arch meets the descending aorta. The second section was taken through the ridge tissue (Figure 3.9).

Histological processing, sectioning, staining and quantification of histological features were performed in the same manner as described earlier for the general aorta histology sample.

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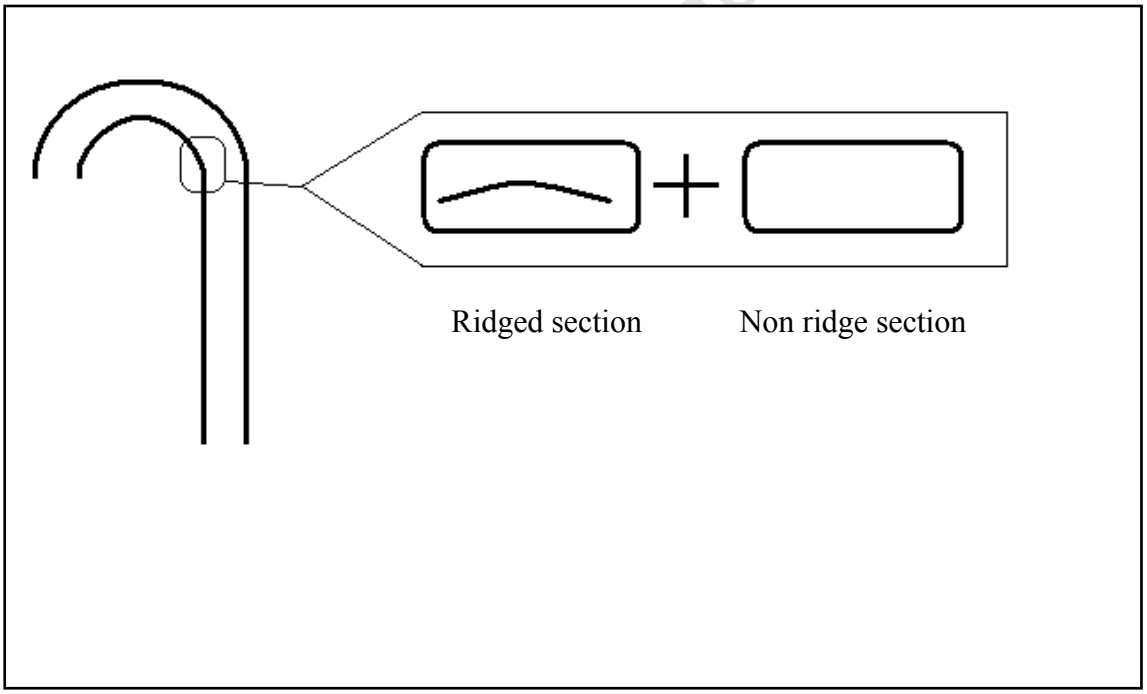

Figure 3.9: Diagram of the aortic arch and abdominal aorta illustrating the position of the ridge and non-ridged sections

3.2.5 Statistical analysis

All statistical analysis was conducted with help from the Department of Statistical Sciences, UCT.

Normality of the sample data was ascertained using the Shapiro-Wilk W Test, using a level of significance of 5%. Parametric analysis was performed on variables found to be normally distributed. The independent t-test was used in order to determine significant differences between mean values as the analysis looked at significant differences different sample sites. Association between variables was determined using the Chi-square test.

If the variables were found not to be normally distributed, the Mann-Whitney U test was used to compare the rank ordering of the variables of the two groups, while the Spearman rank order correlations were used for correlation. All significance levels were at the 5% level unless stated.

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Chapter 4: Results

Of the 71 cadavers dissected in this study, the typical anatomical arrangement of the human aorta and its branches, as described in the literature, was found in 36 individuals (51%). No evidence of variation was found in any of the three aortic regions examined.
In the remaining 35 individuals a range of variations was found.

4.1 Variation in the branching pattern of the human aorta

Variation of the branching pattern along the total length of the aorta was analysed, and is described in 3 sections: variation in the branching pattern of the aortic arch, variation in the branching pattern of the descending aorta, and the gross anatomy of the aortic ridge.

4.1.1 Variation in the branching pattern of the aortic arch

Various types and frequencies of the various branching patterns of the aorta were observed in this study. The presence of these variations differed considerably (Figure 4.1).
Variations included:

- A left vertebral artery arising as a separate branch off the arch of the aorta
- A common brachiocephalic trunk, which comprised a brachiocephalic artery and left common carotid artery
- A right and left brachiocephalic trunk. The right brachiocephalic trunk included the right subclavian and right common carotid arteries. The left brachiocephalic trunk included the left common carotid and the left subclavian arteries.
- A retro-oesophageal right subclavian artery branching as a fourth branch off the arch of the aorta
- A common brachiocephalic trunk, consisting of a brachiocephalic artery and a left common carotid artery, and a common trunk of the left vertebral and left subclavian arteries branching from the arch of the aorta

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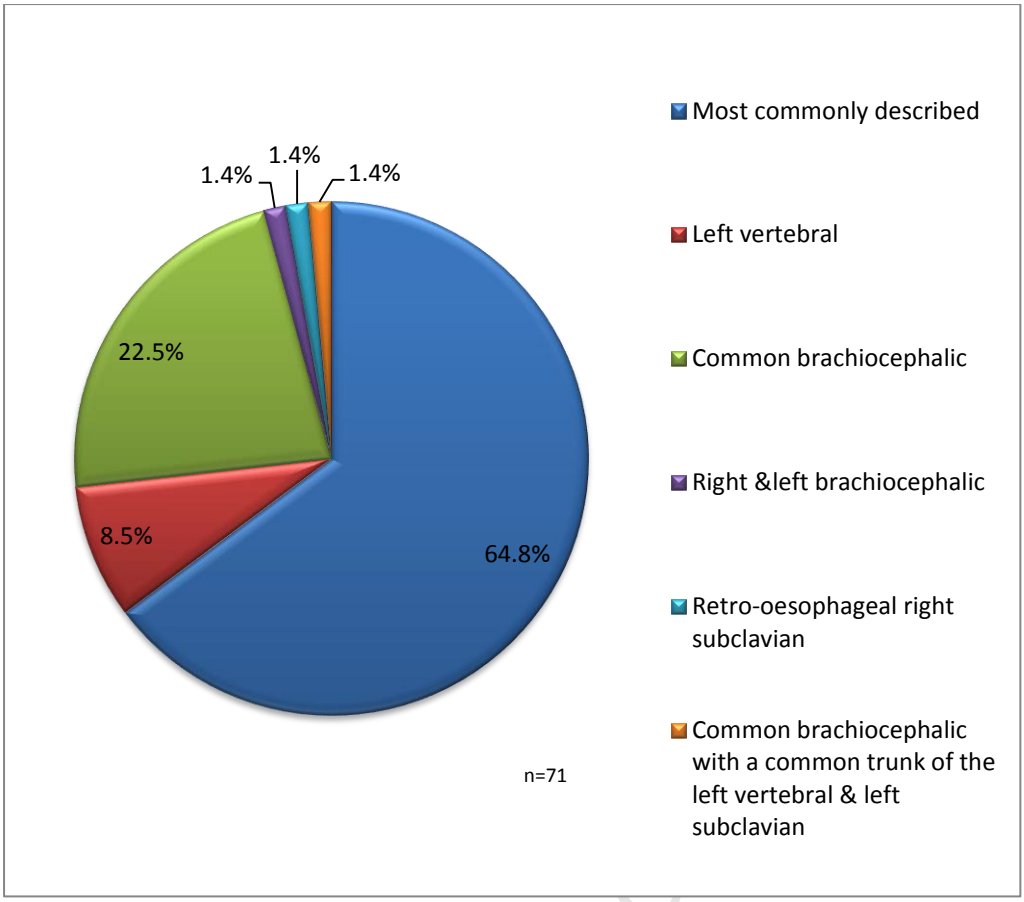

Figure 4.1: Types and the percentages of aortic arch variations found in the UCT cadaver population

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4.1.1.1 Common brachiocephalic trunk

The variation in the common brachiocephalic trunk was observed in 16 cadavers (22.5%). In these cases only 2 arteries arose from the arch of the aorta, instead of the typical 3. The 2 branches are a common brachiocephalic trunk, and the left subclavian artery. The brachiocephalic trunk includes a combined brachiocephalic artery and left common carotid artery (Figure 4.2).

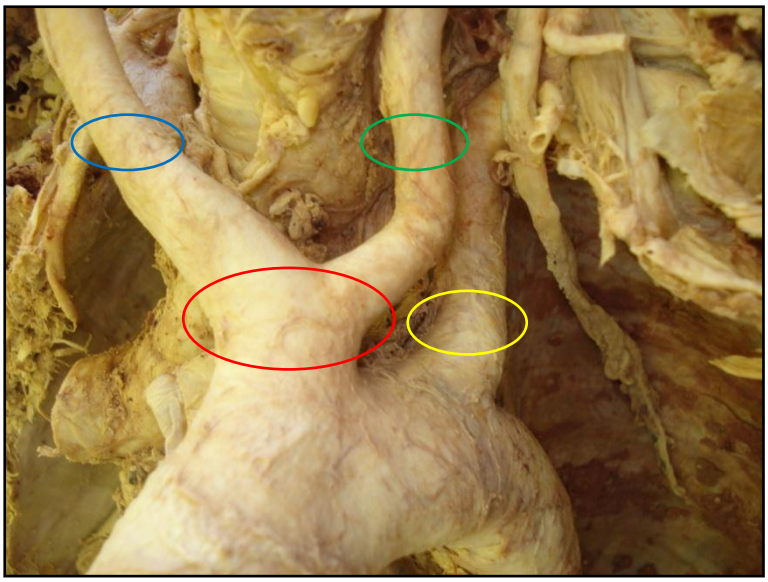

Figure 4.2: A common brachiocephalic trunk (Cadaver number 32/07, anterior view in situ) Red: common brachiocephalic trunk, Yellow: left subclavian artery, Blue: brachiocephalic artery, Green: left common carotid artery

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4.1.1.2 Left vertebral artery

A left vertebral artery arising as a separate branch off the arch of the aorta was observed in 6 of the 71 cadavers (8.5%). In these cases, the left vertebral artery originated from the posterior aspect of the aortic arch between the left common carotid artery and the left subclavian artery (Figures 4.3).

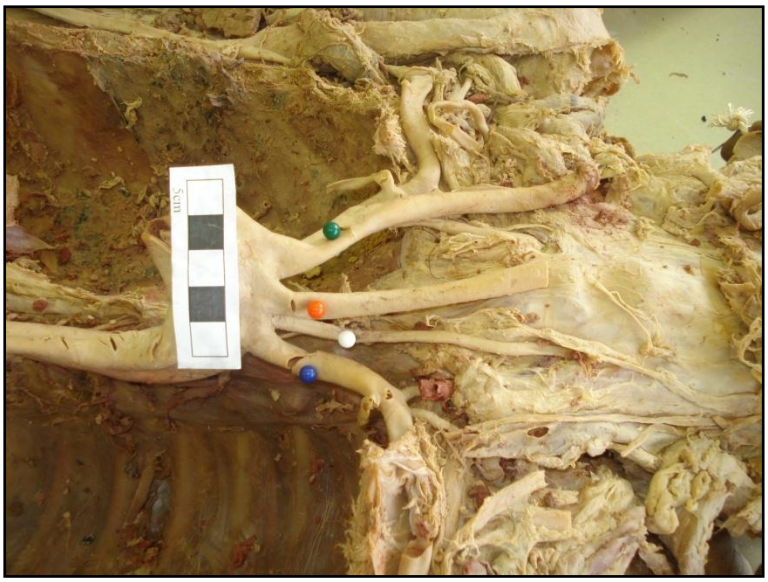

Figure 4.3: A left vertebral artery (Cadaver number 10/08, anterior view in situ)
Green pin: brachiocephalic artery, Red pin: left common carotid, White pin: left vertebral artery, Blue pin: left subclavian artery

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4.1.1.3 Right and left brachiocephalic trunk

Only 1 cadaver (1.4%) had a right and left brachiocephalic trunk. This pattern includes a left brachiocephalic trunk and a right brachiocephalic trunk, and therefore only has 2 branches arising from the aortic arch. The right brachiocephalic artery consists of the right common carotid and the right subclavian arties, while the left brachiocephalic trunk consists of the left common carotid and left subclavian arteries (Figure 4.4).

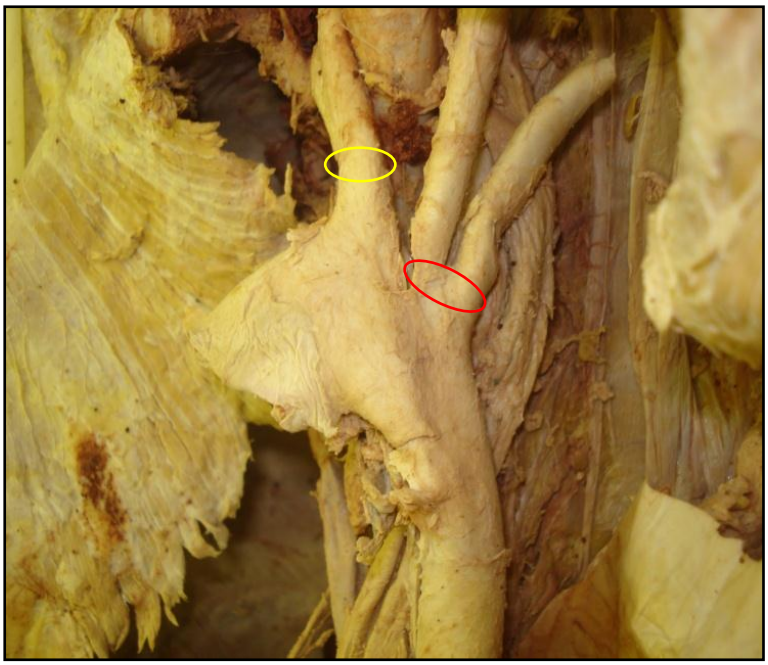

Figure 4.4: Right and left brachiocephalic trunk (Cadaver number 36/08, anterior view in situ)

Yellow: right brachiocephalic trunk, Red: Left brachiocephalic trunk

4.1.1.4 Retro-oesophageal right subclavian artery

This anomaly was observed in a single individual only (1.4%) who was an 86 year old White male. In this individual, the right subclavian artery arises as a separate branch from the posterior aspect of the arch of the aorta. The vessel was traced and was found to run posterior to the oesophagus towards the right axilla (Figures 4.5 and 4.6).

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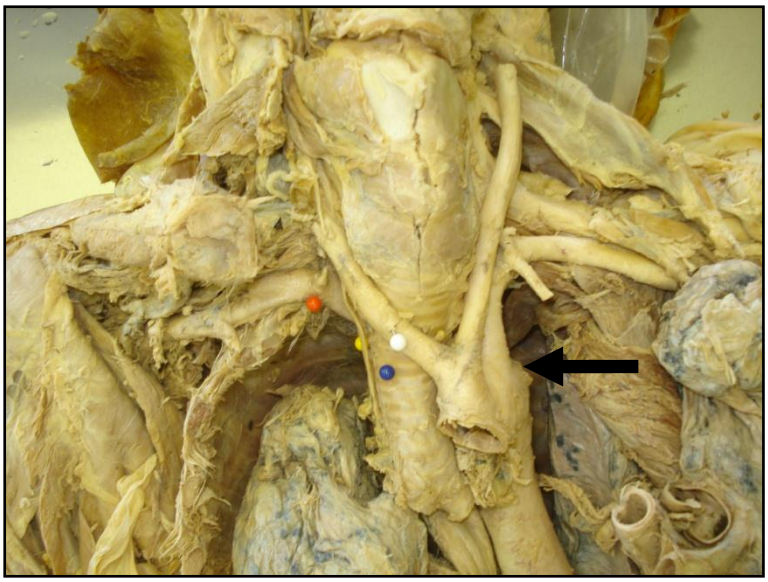

Figure 4.5: Retro-oesophageal right subclavian artery (Cadaver number 08/07, anterior view in situ)

Red pin: right subclavian artery, Yellow pin: oesophagus, Blue pin: trachea,

White pin: right common carotid artery, Black arrow: origin or right subclavian artery

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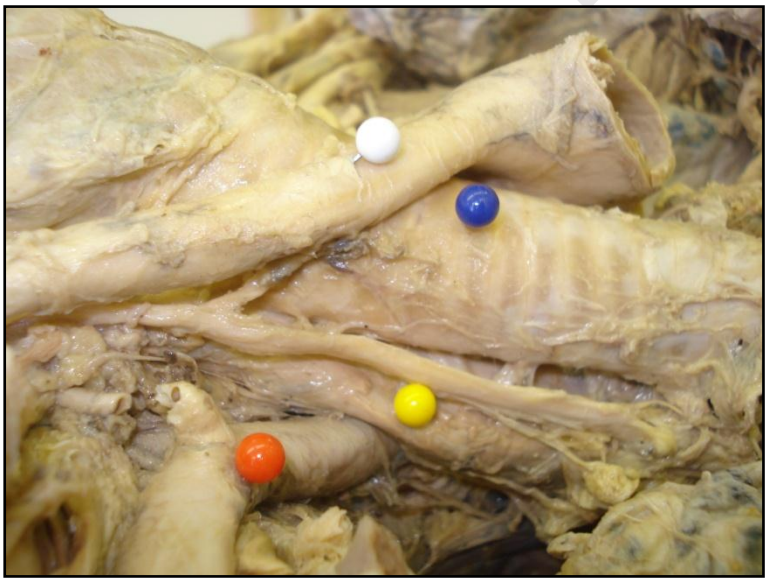

Figure 4.6: Retro-oesophageal right subclavian artery (Cadaver number 08/07, lateral view in situ)

Red pin: right subclavian artery, Yellow pin: oesophagus, Blue pin: trachea,

White pin: right common carotid artery

4.1.1.5 Common brachiocephalic trunk, with a common origin for the left vertebral and left subclavian arteries

This pattern was found in a single individual only 1.4%. In this case the pattern includes 2 branches off the aortic arch; a brachiocephalic trunk and a trunk in which the left vertebral and left subclavian artery arise together off the aortic arch together. The common brachiocephalic trunk consists of a brachiocephalic artery and a left common carotid artery (Figure 4.7).

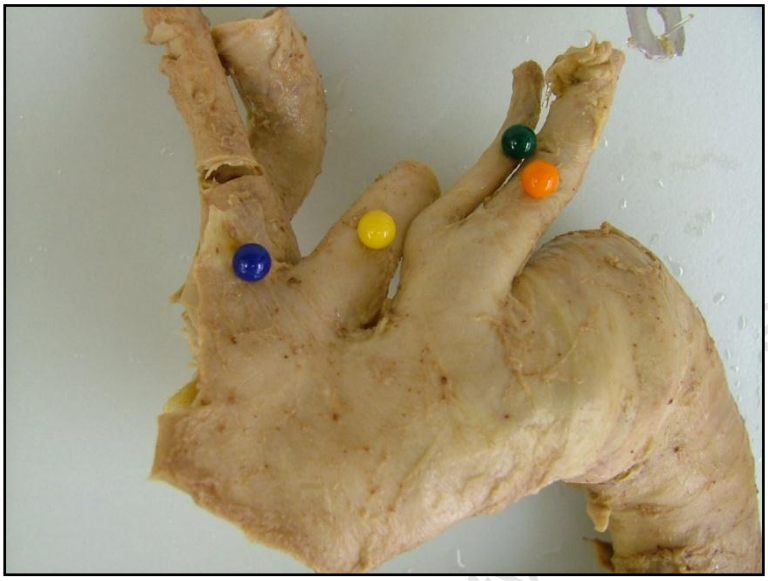

Figure 4.7: Common brachiocephalic trunk, with a common origin for the left vertebral and left subclavian arteries (Cadaver number 30/07, anterior view)
Blue pin: brachiocephalic artery, Yellow pin: left common carotid artery, Green pin: left vertebral artery, Orange pin: left subclavian artery

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4.1.2 Variation in the branching pattern of the descending aorta

Three different variations in the branching pattern of the descending aorta were observed. These included:

1) Supernumerary posterior intercostals arteries
2) Duplicated renal arteries
3) Coeliacomesenteric artery

Note that no gonadal variation was observed.

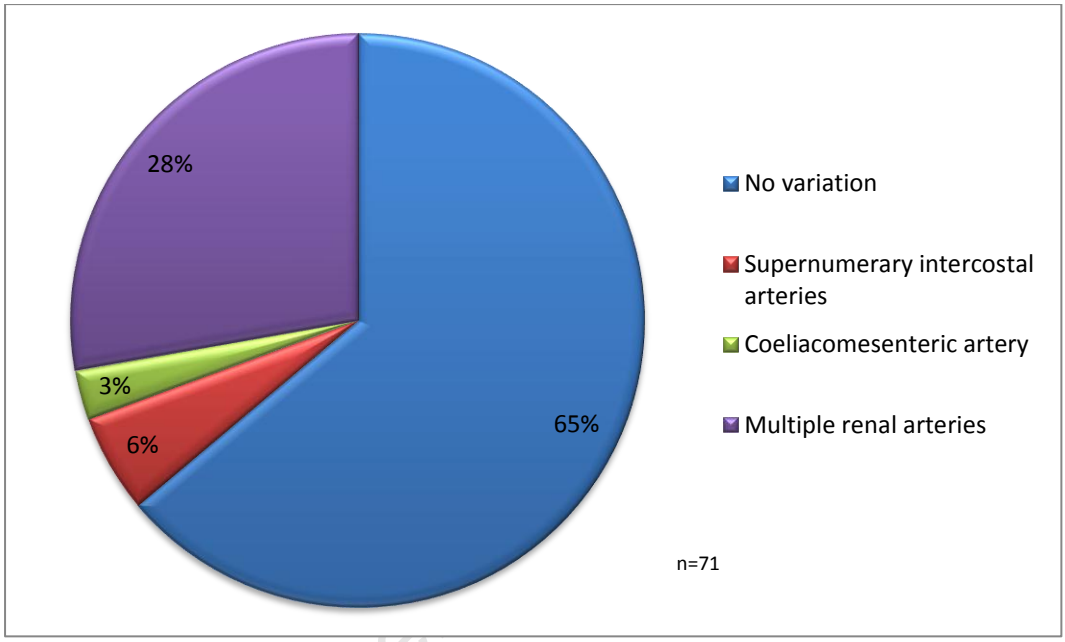

Figure 4.8: Number of cadavers with each type of descending aorta variation
Note that the numbers do not total 71 as there were individuals with more than one variation

4.1.2.1 Supernumerary posterior intercostal arteries

Supernumerary posterior intercostal arteries were observed in 4 (6%) individuals. Two individuals had an additional pair of intercostal arteries, while another two individuals only had an additional artery on the left side of the proximal descending thoracic aorta. The origin of the additional arteries in all 4 individuals was proximal to the third posterior intercostal arteries on the posterior surface of the descending thoracic aorta (Figure 4.9).

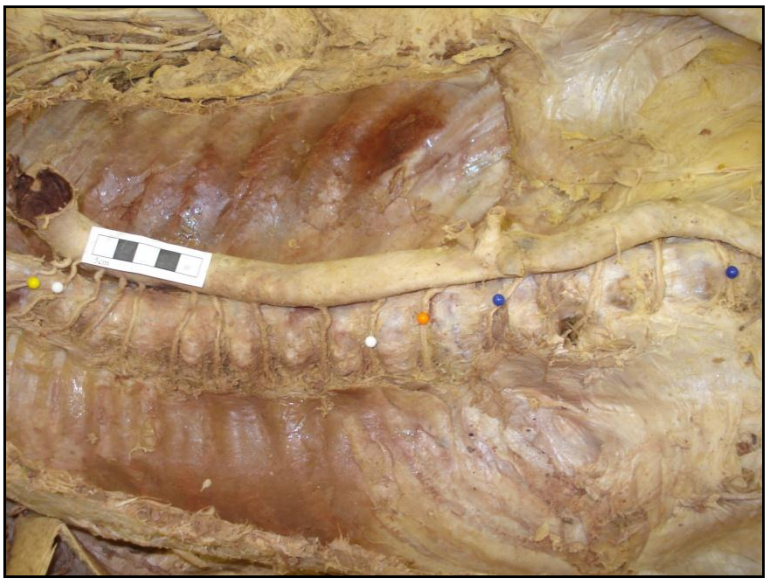

Figure 4.9: Addition posterior intercostal arteries (yellow pin, Cadaver number 18/07, anterior view in situ; cranial end left of photo, caudal end right of photo)
White pins: 3rd and 11th right posterior intercostal arteries, Orange pin: right subcostal artery, Blue pins: 1st and 4th right lumbar arteries

4.1.2.2 Multiple renal arteries

Of the 71 cadavers dissected, 20 individuals (28%) had multiple renal arteries. The origins of these multiple arteries varied in position along the abdominal aorta (Figures 4.10 and 4.11). Ten individuals had duplicate vessels originating from the left and ten from the right side of the abdominal aorta.

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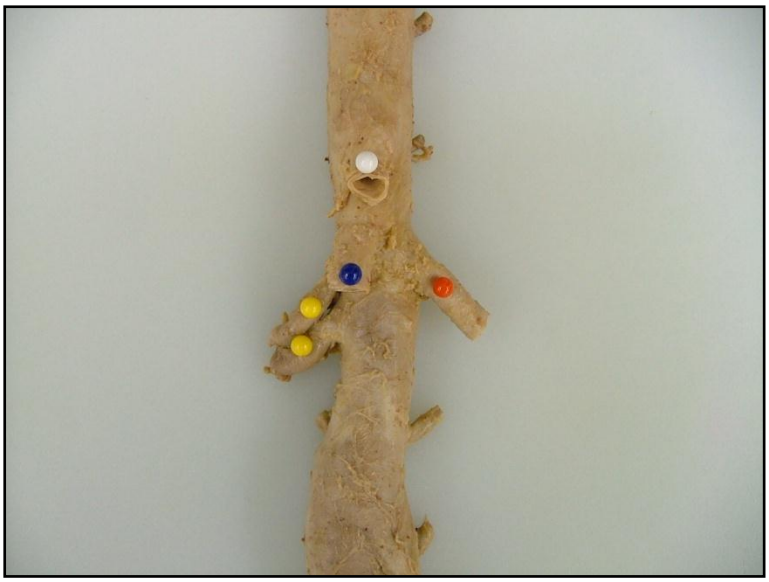

Figure 4.10: Multiple renal arteries arising from the right side of the aorta (Cadaver number 32/07 anterior view)

Note the origin of the additional renal artery immediately distal to the right renal artery

White pin: coeliac trunk, Blue pin: superior mesenteric artery, Red pin: left renal artery, Yellow pins: multiple right renal arteries

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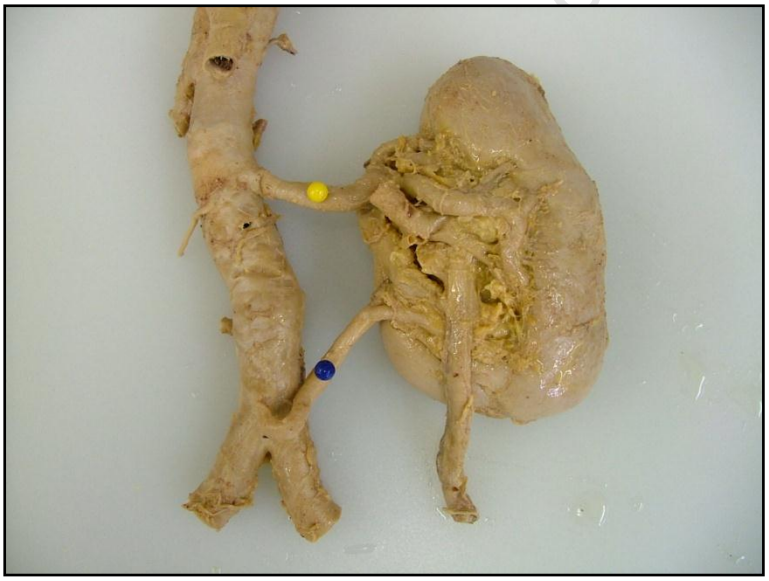

Figure 4.11: Multiple renal arteries arising from the left side of the aorta (Cadaver number 04/07, anterior view)

Note the distal site of origin of the additional renal artery (blue pin), at the bifurcation of the aorta

Yellow pin: left renal artery

4.1.2.3 Coeliacomesenteric artery

A combined coeliac trunk and superior mesenteric artery was found in 2 (or 3% of) individuals (Figure 4.12).

Figure 4.12: A common coeliacomesenteric artery (black ring, Cadaver 81/07 lateral view) Yellow pin: coeliac trunk, Red pin: superior mesenteric artery, Blue pin: inferior mesenteric artery

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4.1.3 Gross anatomy of the aortic ridge

The luminal surface of the mortuary samples was assessed for any signs of macroscopic variation. During the examination of these vessels a pronounced curved ridge-like structure and indentation on the luminal surface of the aortic vessel wall was observed at the junction of the aortic arch and descending aorta (Figures 4.13 and 4.14).

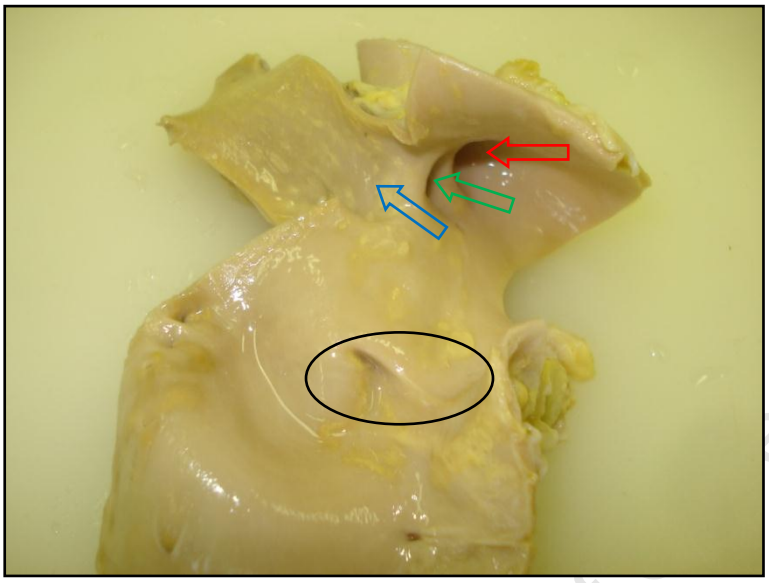

Figure 4.13: Photograph of the aortic ridge (black ring) in a 27 year old Black male (posterior view)
Red arrow: brachiocephalic trunk origin off aortic arch,
Green arrow: left common carotid artery origin off aortic arch,
Blue arrow: left subclavian artery origin off aortic arch (dissected open)

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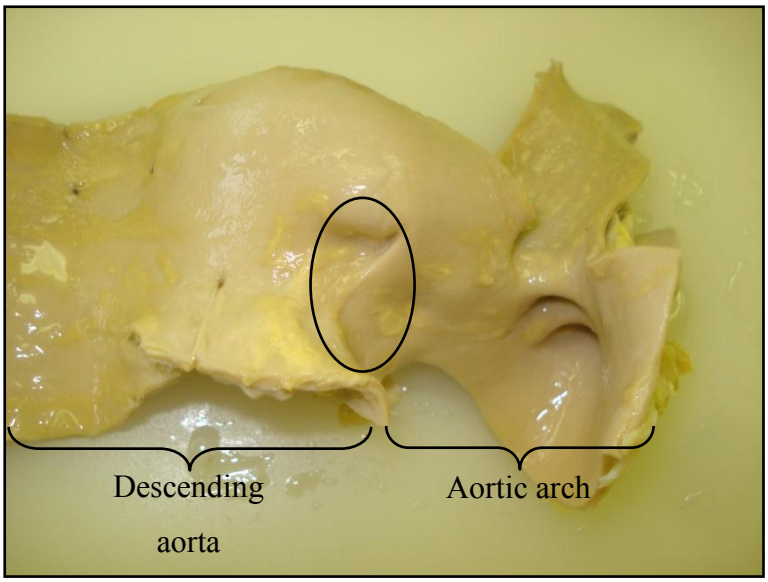

Figure 4.14: Photograph of the aortic ridge (black ring) indicating its position along the aorta in a 27 year old Black male (posterior view of the same specimen in Figure 4.13)

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4.2 Statistical analysis of the gross anatomic variation

4.2.1 Total of variations

A variation in the branching pattern along the total length was noted as the presence of either an aortic arch variation or a descending aorta variation or the combination of both. The variation along the length of the aorta was assessed according to sex and race. Of the 71 cadavers dissected, 35 (49%) had evidence of a variation in branching pattern (Figure 4.15).

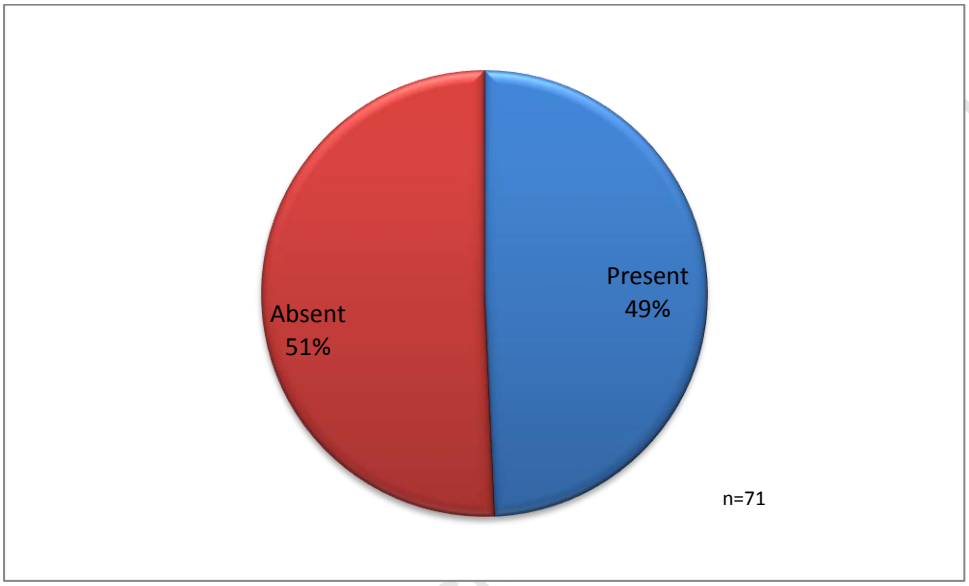

Figure 4.15: Presence of variation in the branching pattern of the aorta in the UCT cadaver population

4.2.1.1 Sex and total of variations

With 52 male cadavers and only 19 female cadavers, caution must be taken when comparing results between male and female cadavers. The percentage of variation along the aorta was 48% in males and 53% in females. These levels of variation in the branching pattern were approximately equal in male and female cadavers, thus no association between presence of variation and sex of the individuals was found (Chi-square 0.69, p=0.79). Figure 4.16 below indicates the percentages of male and female cadavers in which variation in the branching pattern of the aorta was observed.

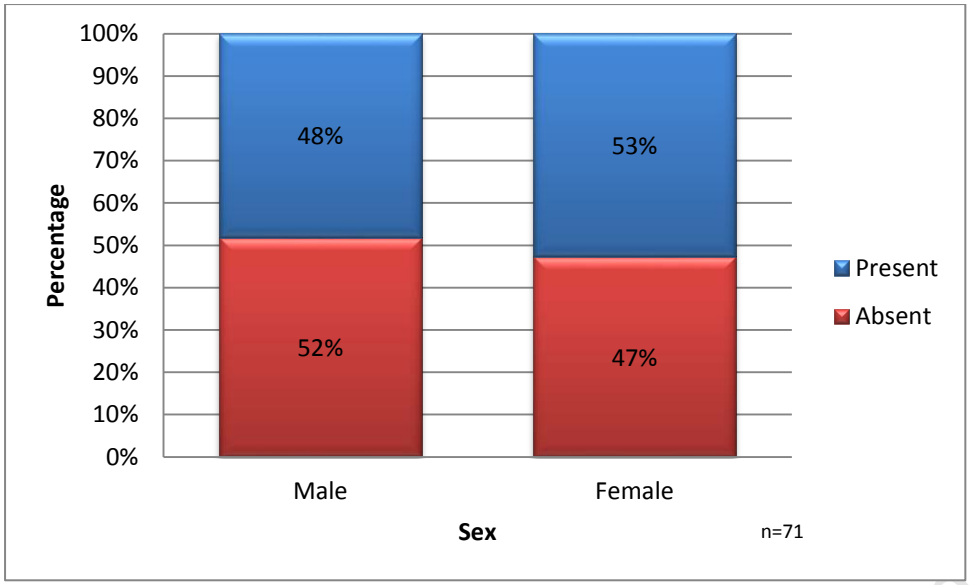

Figure 4.16 Percentage of male and female cadavers with variation in the branching pattern of the aorta

4.2.1.2 Race and total variation

Total variation was assessed for each racial category. The results from Figure 4.17 indicate the percentage of variation of the branching pattern along the aorta in each racial category. Variation was most frequent in Coloured cadavers (57%) and less frequent in White and Black cadavers with 50% and 40% respectively, however, there were limited numbers of individuals in the Black and Coloured racial categories and caution should be taken when analysing these results. Although there were limited individuals in each racial category, there was no association found between variation along the length of the aorta and race (Chi-square 2.89, p=0.24).

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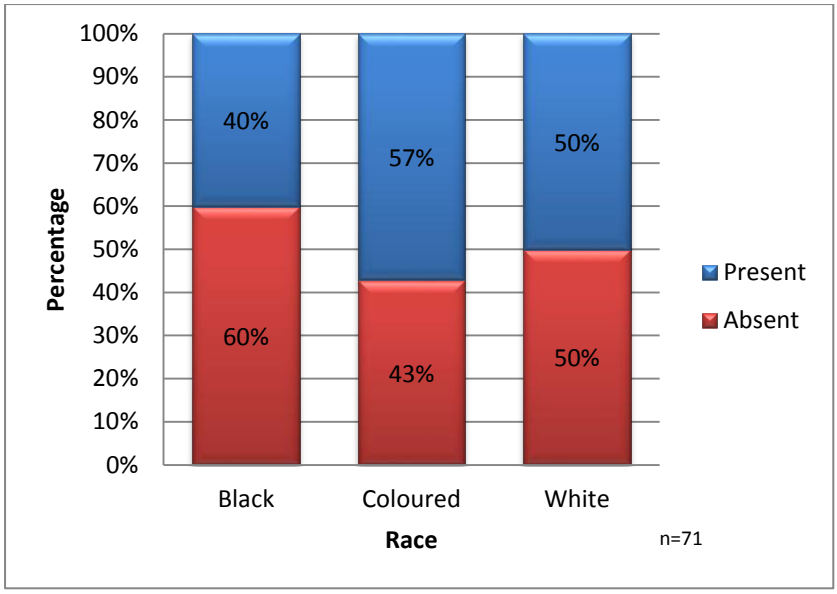

Figure 4.17: Percentage of cadavers with variation in the branching pattern of the aorta in each racial category

4.2.2. Variation in the branching pattern of the aortic arch

Of the 71 cadavers dissected, 25 cadavers or 35% had evidence of a variation in the branching pattern of the aortic arch (Figure 4.18). Note that an absent variation was considered as an arch with 3 branches: a brachiocephalic artery, a left common carotid artery and a left subclavian artery.

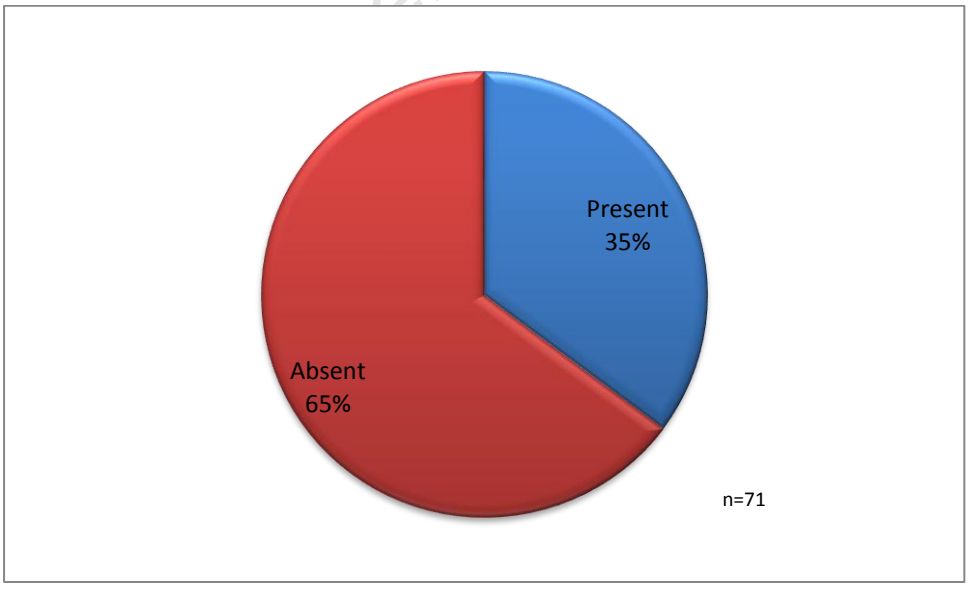

Figure 4.18: Presence of aortic arch variation in the UCT cadaver population

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4.2.2.1 Sex and variation in the branching pattern of the aortic arch

Variation in the branching pattern of the aortic arch was assessed according to sex. Figure 4.19 indicate the percentages of male and female cadavers with an aortic arch variation. Although the percentages indicate that the male cadavers had a higher aortic arch variation than the female cadavers, there was no association found between presence of aortic arch variation and sex (Chi-square 0.65, p=0.42).

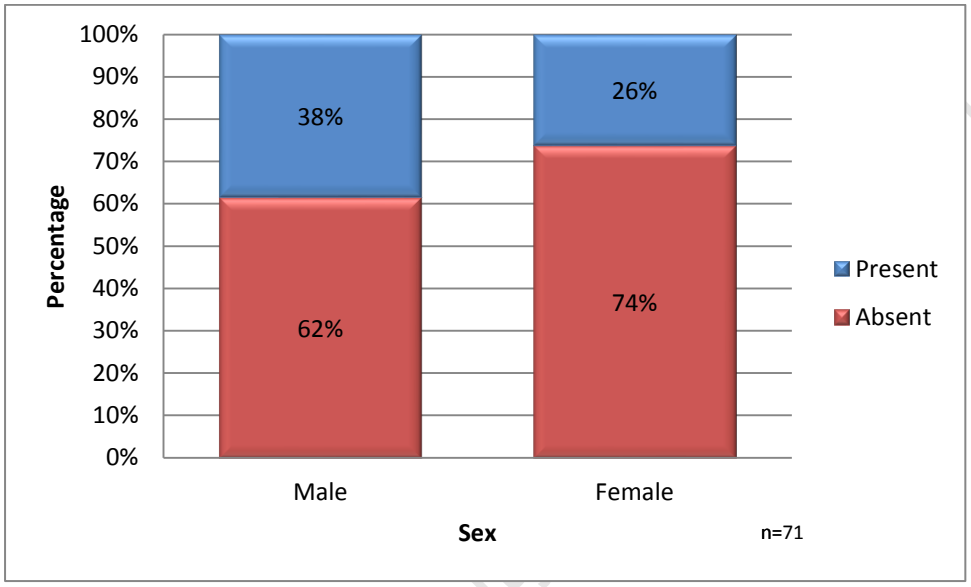

Figure 4.19: Percentage of male and female cadavers with aortic arch variation

4.2.2.2 Race and aortic arch variation

Aortic arch variation was assessed with respect to race. Figure 4.20 indicates that aortic arch variation occurs more frequently in Coloured cadavers (50%). Aortic arch variation was less frequent in Black and White cadavers with 33% and 31% respectively. There was no association between presence of aortic arch variation and race (Chi squared 2.16, p=0.34).

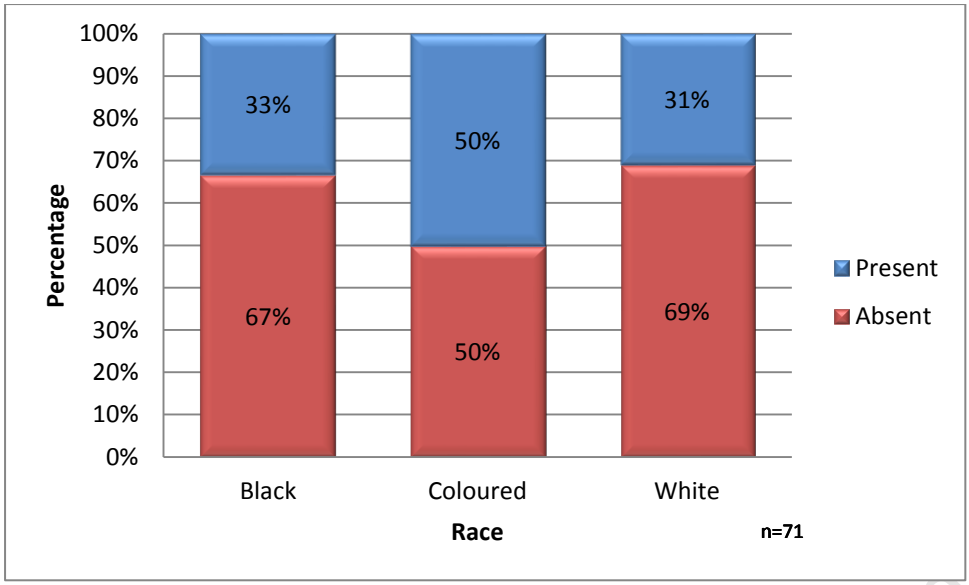

Figure 4.20: Percentage of cadavers present with aortic arch variation in each racial category

4.2.3 Variation in the branching pattern of the descending aorta

Variations in branching patterns of the descending aorta were documented during the dissection of the 71 cadavers and were assessed according to sex and race. Of the 71 cadavers, 25 cadavers (35%) of the population had evidence of variation in the descending aorta (Figure 4.21).

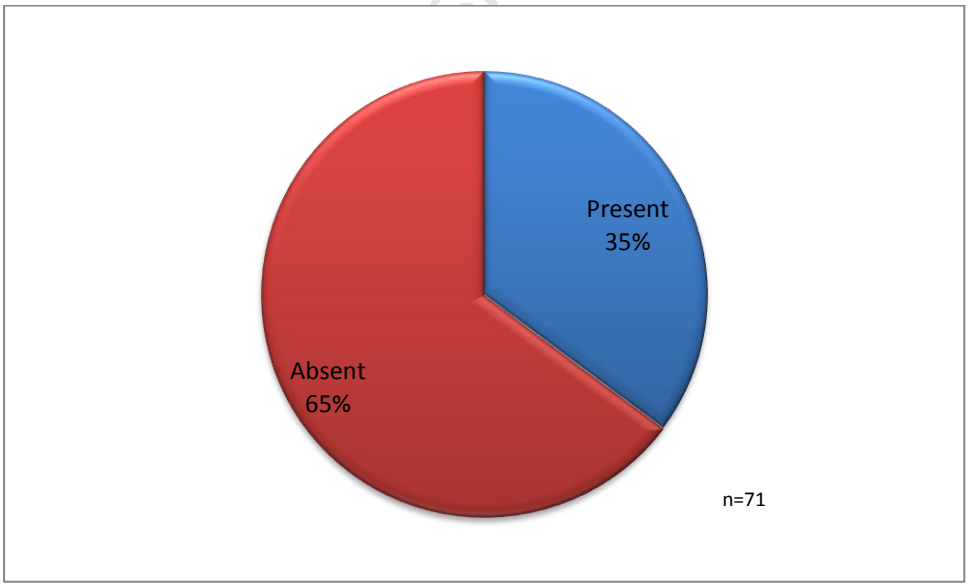

Figure 4.21: Presence of variation along the descending aorta in the UCT cadaver population

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4.2.3.1 Sex and descending aorta variation

Variations in the branching patterns of the descending aorta in the cadaver population were analysed with respect to sex. Figures 4.22 indicate the percentages of male and female cadavers in which variation was observed. These results indicate that 42% of the female cadavers and 33% of the male cadavers showed some sort of variation. There was no association found between presence of variation along the descending aorta and sex (Chi-square 0.54, p=0.46).

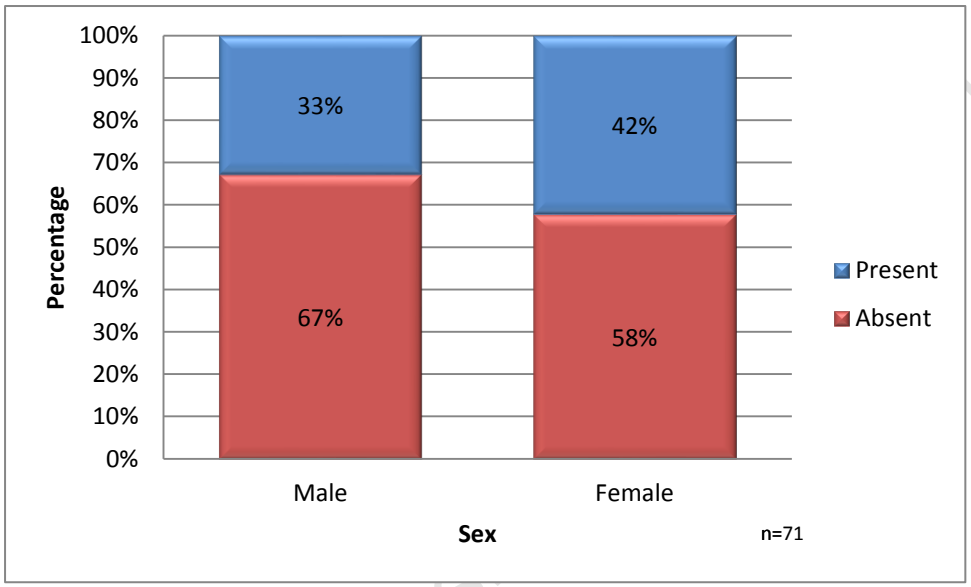

Figure 4.22: Percentage of male and female cadavers with abdominal aorta variation

4.2.3.2 Race and descending aorta variation

Variation in the descending aorta was also assessed for each racial category (Figure 4.23). These results indicate that variation along the descending aorta was observed most frequently in Coloured cadavers (43%). Variation was less frequent in Black and White cadavers with a frequency of 33% each. There was no association found between presence of descending variation and race (Chi-square 0.45, p=0.80).

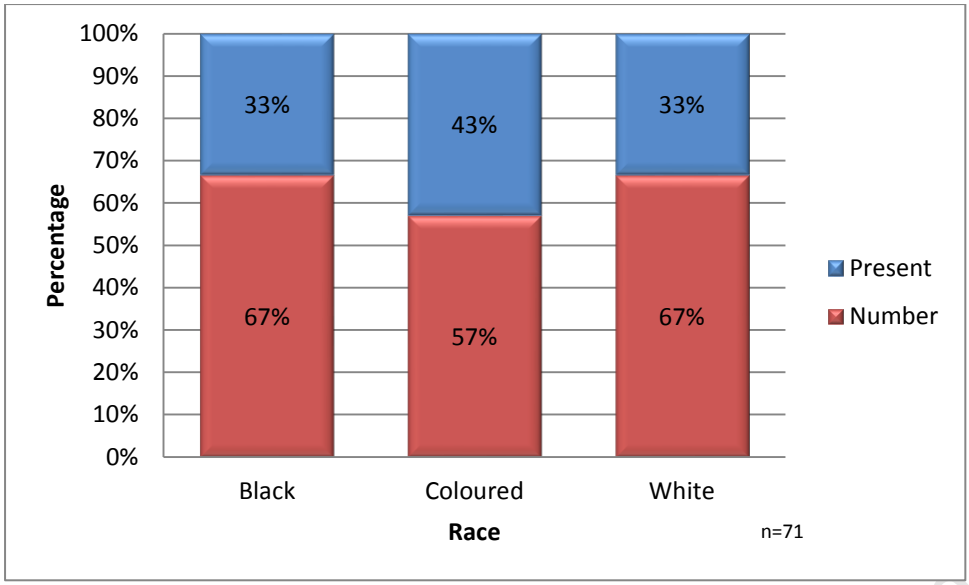

Figure 4.23: Percentage of cadavers present with abdominal aorta variation with respect to race

4.2.4. Prevalence of the aortic ridge

The aortic ridge as described above was observed in 111 of the 149 mortuary specimens examined. Figure 4.24 below illustrates the observed frequency of the presence of the aortic ridge.

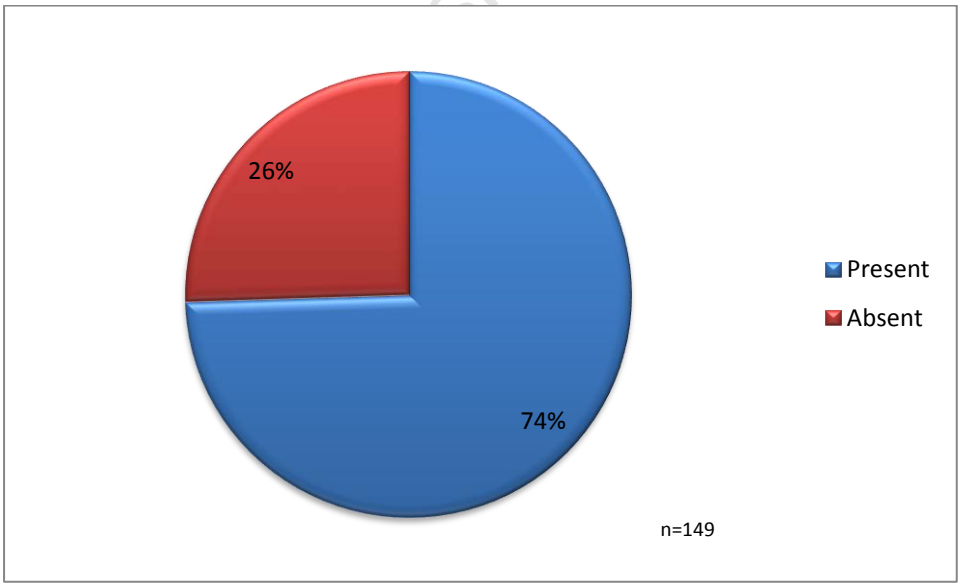

Figure 4.24: Presence of the aortic ridge in the mortuary sample

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4.2.4.1 Sex and presence of aortic ridge

The presence of the aortic ridge in males and females was compared. Figures 4.25 and 4.26 indicate the numbers and percentages respectively of males and females in whom the ridge was present. These results should be viewed with caution as there were fewer females in the sample. The results indicate that 92 males and 19 females had the ridge present (Figure 4.25), while Figure 4.26 illustrates that 76% of the male population and 68% of the female population had the aortic ridge present. While there were a larger percentage of males with the ridge present, no association between presence of the aortic ridge and sex was found (Chi-square 0.80, p=0.37).

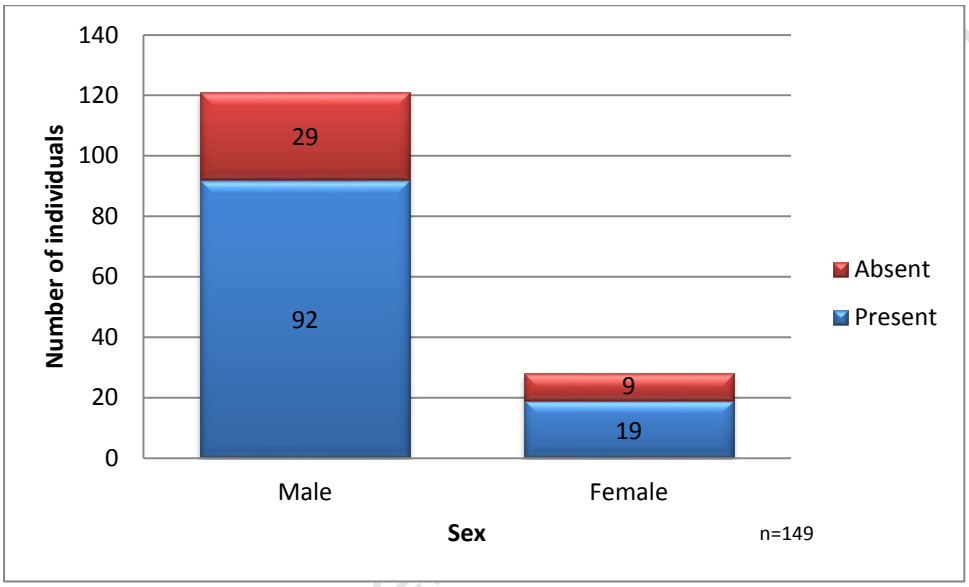

Figure 4.25: Number of males and females present with the aortic ridge

Unive

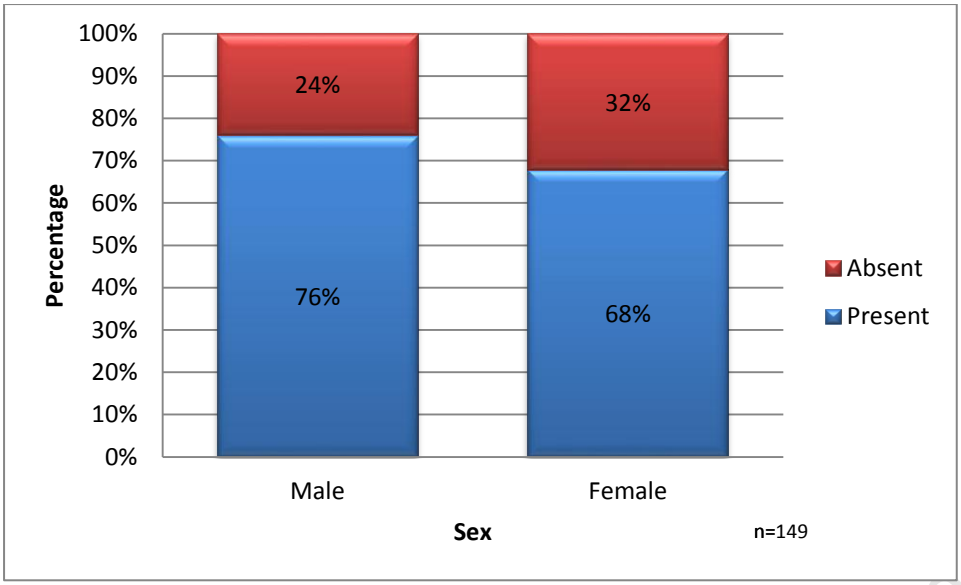

Figure 4.26: Percentage of males and females present with the aortic ridge

4.2.4.2 Race and presence of aortic ridge

The presence of the aortic ridge was assessed in each racial category. Figure 4.27 indicates the number of individuals observed with an aortic ridge in each racial category. Caution should be taken when interpreting these results due to fewer numbers of individuals in the Coloured and White racial categories. The results illustrated in Figure 4.28 indicate that the ridge was present most frequently in Black individuals (81%). The ridge was present less frequently in White and Coloured individuals (67% and 66% respectively). An association between the presence of the ridge and race of the individuals was not significant at a 5% level, however it was significant at a 10% level (Chi-square 4.56. p=0.10).

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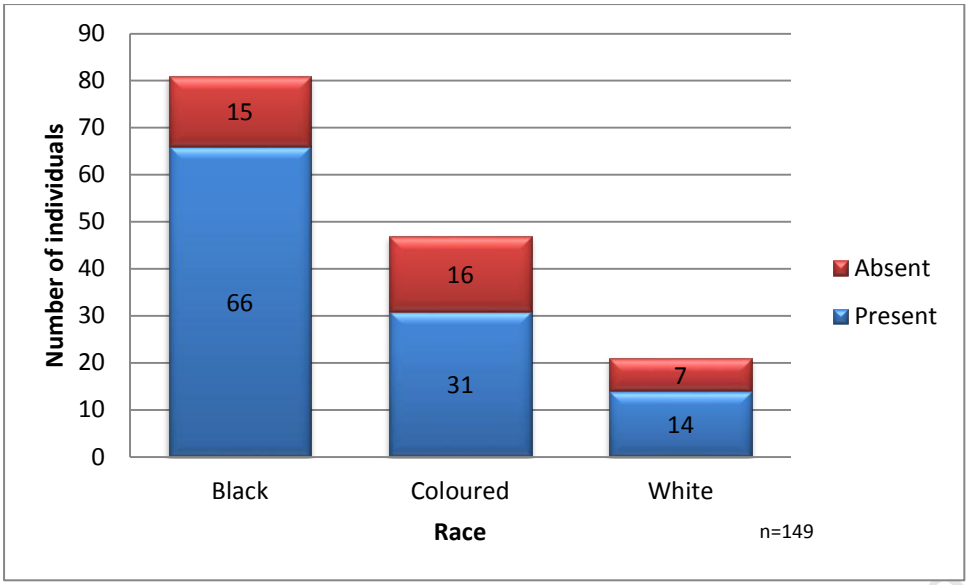

Figure 4.27: Number of individuals present with the aortic ridge in each racial category

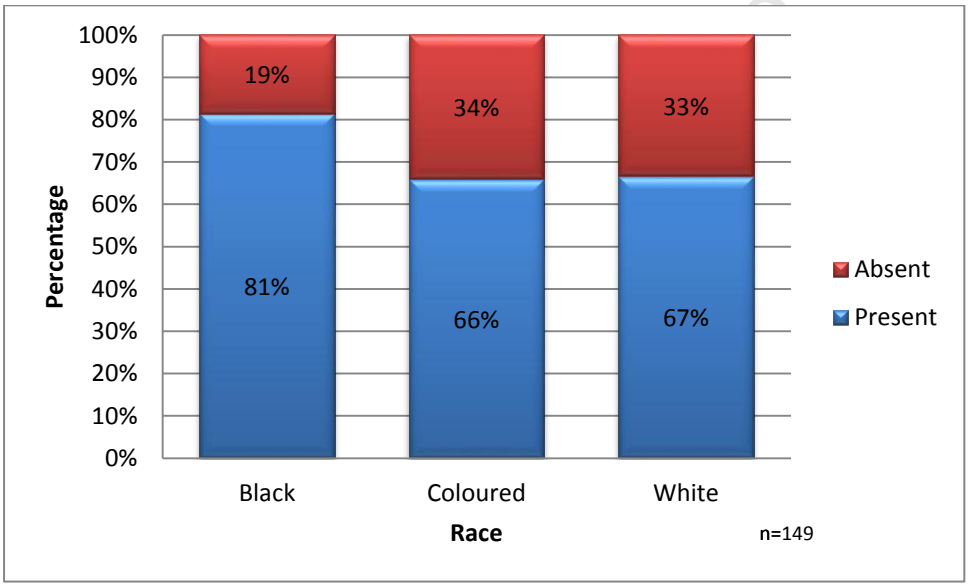

Figure 4.28: Percentage of individuals present with the aortic ridge in each racial category

4.2.4.3 Age and presence of aortic ridge

The presence of the aortic ridge was assessed in age groups 0-19 years, 20-39 years, 40-59 years and those older than 60 years. Caution should be taken when interpreting these results due to less than 5 individuals in certain age groups.

Figures 4.29 and 4.30 indicate the numbers and percentages respectively of individuals in each age group in whom the aortic ridge was present. Figure 4.30 shows that the aortic ridge

is most frequently observed in individuals younger than 20 years (85%) and individuals in the age group 20-39 years (80%), whilst only 58% of individuals in the 40-59 age group and 60% of individuals in the 60+ age group had the ridge present. The association between presence of the aortic ridge and age of the individuals was significant at the 10% level, and approached significance at the 5% level (Chi-square 4.57, p=0.056).

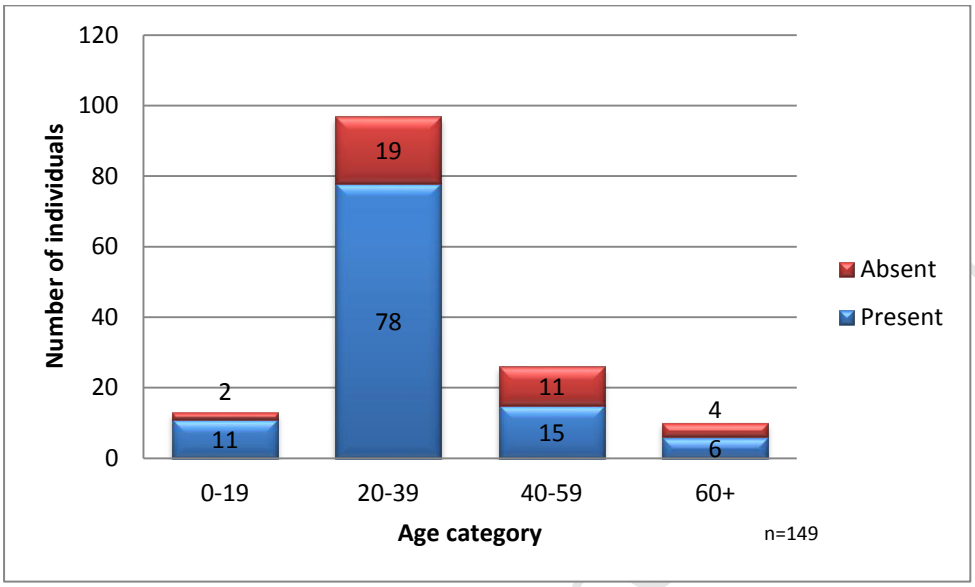

Figure 4.29: Number of individuals present with the aortic ridge in each age group

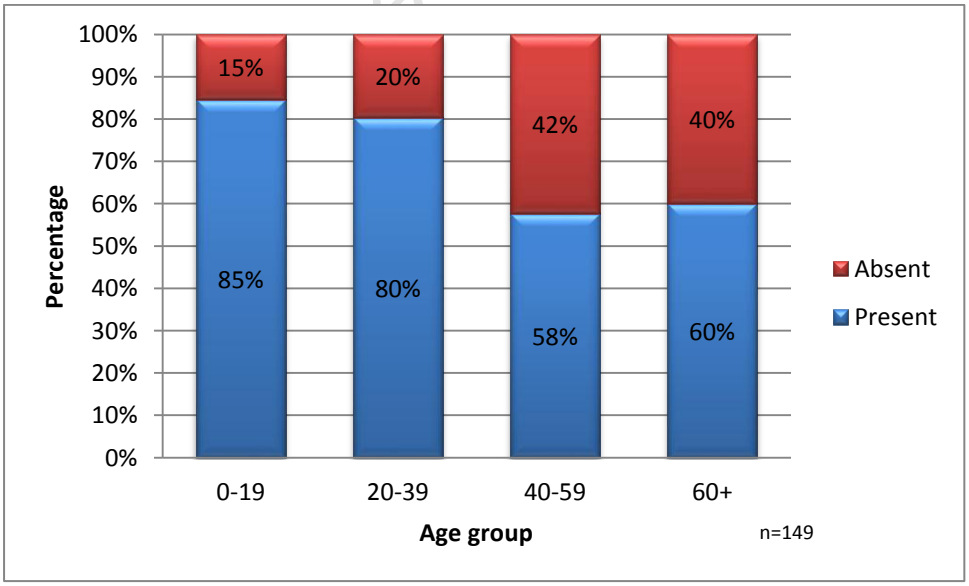

Figure 4.30: Percentage of individuals present with the aortic ridge in each age group

The average age was assessed for those individuals with the aortic ridge present and those without (Figure 4.31). The average age for those with the ridge present was 32.2 years, while those without the ridge present was 40.7 years. Figure 4.32 illustrates the distribution of age between the two groups. There was a significant difference between the average ages of the individuals with the ridge present and those with the ridge absent (t=0.90, p=0.001).

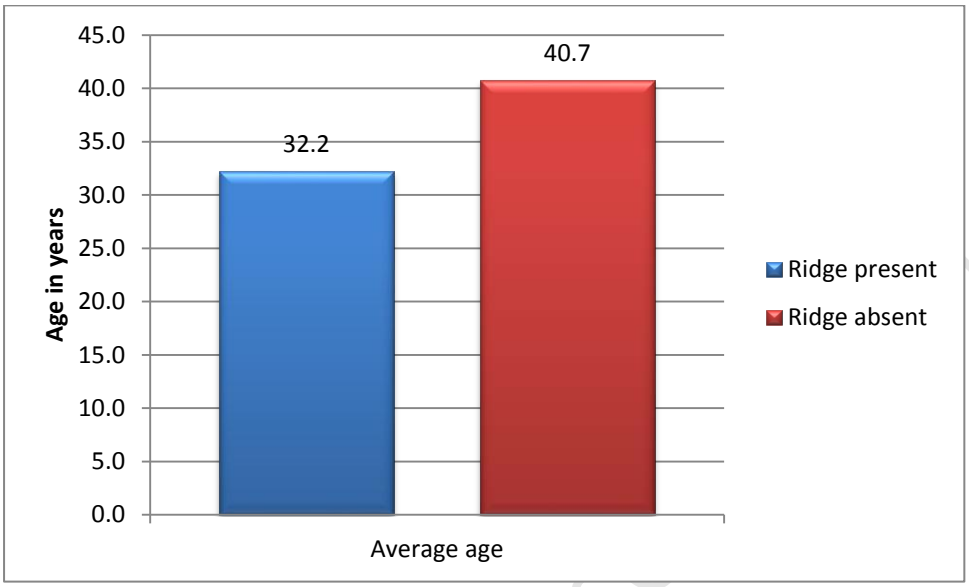

Figure 4.31: Average age of individuals with and without the aortic ridge present

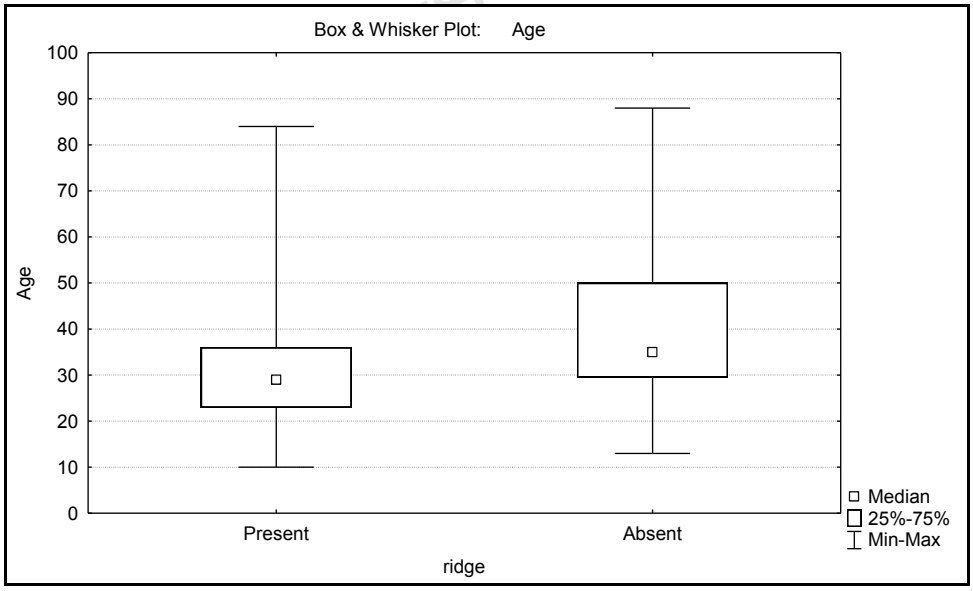

Figure 4.32: Box & Whisker plot indicating the distribution of age for the individuals with and without the aortic ridge present

4.3 Histological variation

4.3.1 Histological variation of the aorta and branching and non-branching sites

The results of the investigation of the histological structure at different sites along the aorta appear below. Histological features investigated and analysed were the presence of acid mucopolysaccharides, tunica intima and media measurements, intimomedial ratios and elastin fragmentation.

4.3.1.1 Acid mucopolysaccharides

Presence of acid mucopolysaccharides based on positive AB-PAS staining for each of the 4 regions along the aorta was recorded. Note that for the analysis of acid mucopolysaccharides, the two renal sites were combined as were the two bifurcation sites due to their close proximity. Acid mucopolysaccharides were found to be abundant (Figure 4.33). With the exception of the ascending site and the anterior intercostal site in a single individual, acid mucopolysaccharides were found in all individuals at all four sites. Figure 4.34 depicts the presence of acid mucopolysaccharides at each sample site and the presence of acid mucopolysaccharides at branching and non-branching sites.

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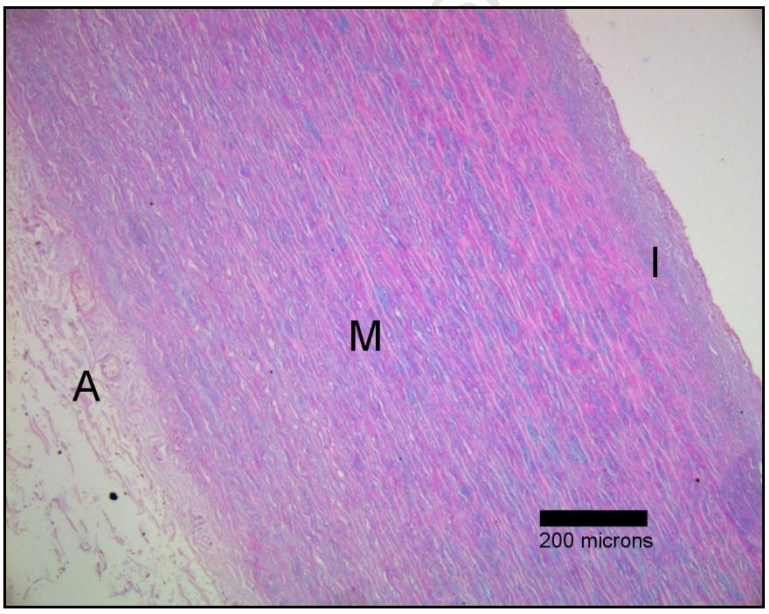

Figure 4.33: Micrograph indicating acid mucopolysaccharides (blue) in the ascending aorta of a 21 year old Black male (AB-PAS stain)
I: Tunica intima, M: Tunica media, A: Tunica adventitia

Note the intense positive staining in the tunica media and the lighter positive staining in the tunica intima. In addition, note that the orientation of the acid mucopolysaccharides in between the lamella units of the tunica media

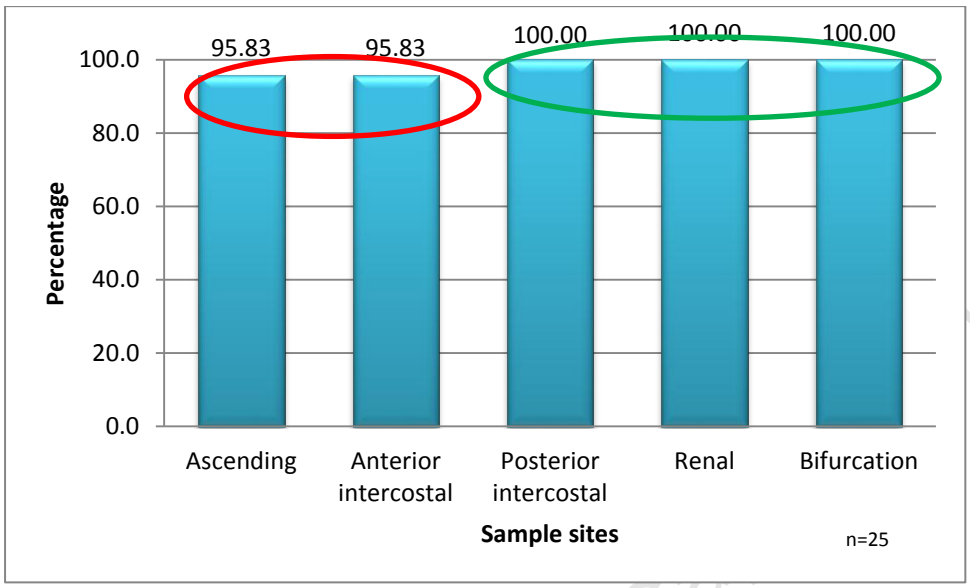

Figure 4.34: Comparison of presence of acid mucopolysaccharides in branching and non-branching sites

Note red ring indicating non-branching sites and green ring indicating branching sites

4.3.1.2 Vessel wall measurements

Average tunica intima thickness

As described in Chapter 4, the thickness of the tunica intima was measured at 3 locations to obtain an average measurement at each site for the 25 aortae. The 25 average tunica intima measurements at each site were then averaged. Figure 4.35 illustrates that the thickest average tunica intima was 335 microns at the bifurcation tip site and the thinnest average tunica intima was 190 microns at the ascending sample site.

University of Ca

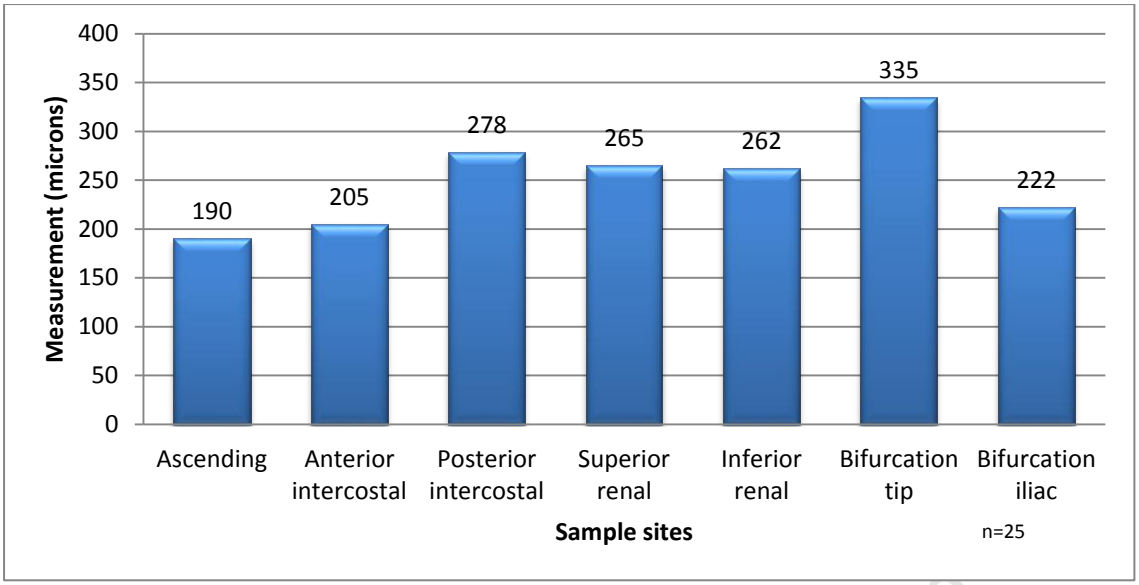

Figure 4.35: Average thickness (μ) of the tunica intima at each of the 7 sites along the aorta

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Average tunica media thickness

The thickness of the tunica media was measured as described above and the average tunica media measurement was calculated for the 7 sites for the 25 aortae (Figure 4.36). The tunica media was found to be widest at the ascending aorta site (at 1357μ), and narrowest at the inferior renal site (at 989μ).

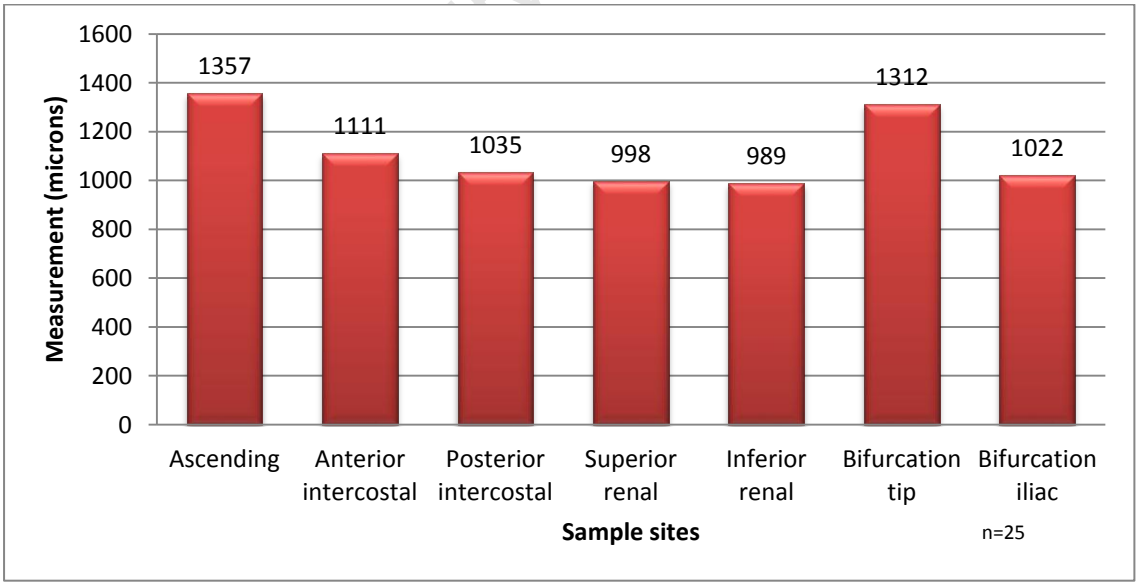

Figure 4.36: Average thickness (μ) of the tunica media at each of the 7 sites along the aorta

Average tunica measurements

The trend of the average tunica thickness along the length of the aorta can be investigated from the results below (Figure 4.37). These results indicate no obvious trend in the tunica measurements proximal to distal along the length of the aorta. Two decreased measurements were observed in the tunica intima at the ascending and anterior intercostal sites, while two increased measures can be noted in the tunica media measurements at the ascending and bifurcation tip sites.

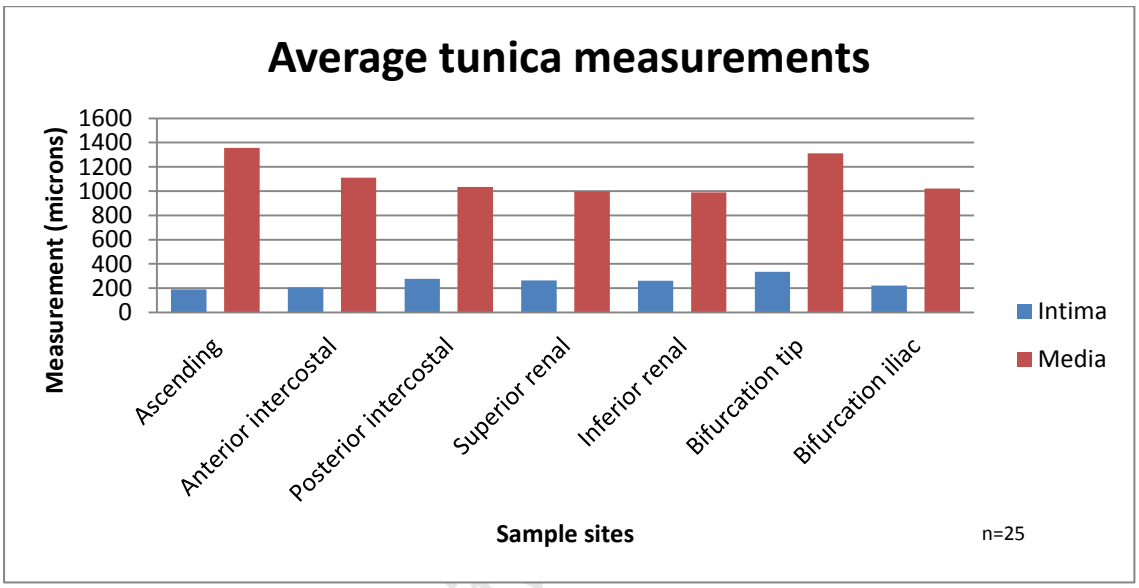

Figure 4.37: Average tunica thickness (µ) at each of the 7 sites along the aorta

Comparison of branching and non-branching sites

The average thickness of the tunica intima and media, at branching and non-branching sites, were compared. The results (Figure 4.38 and 4.39) indicate that the average thickness of the tunica intima is lower at the non-branching sites than at the branching sites. The average tunica intima thickness for the non-branching sites was 198µ and for the branching sites was 272 µ. There was a statistically significant difference between the average tunica intima measurements at the branching and non-branching sites (t=3.89, p=0.0001).

University

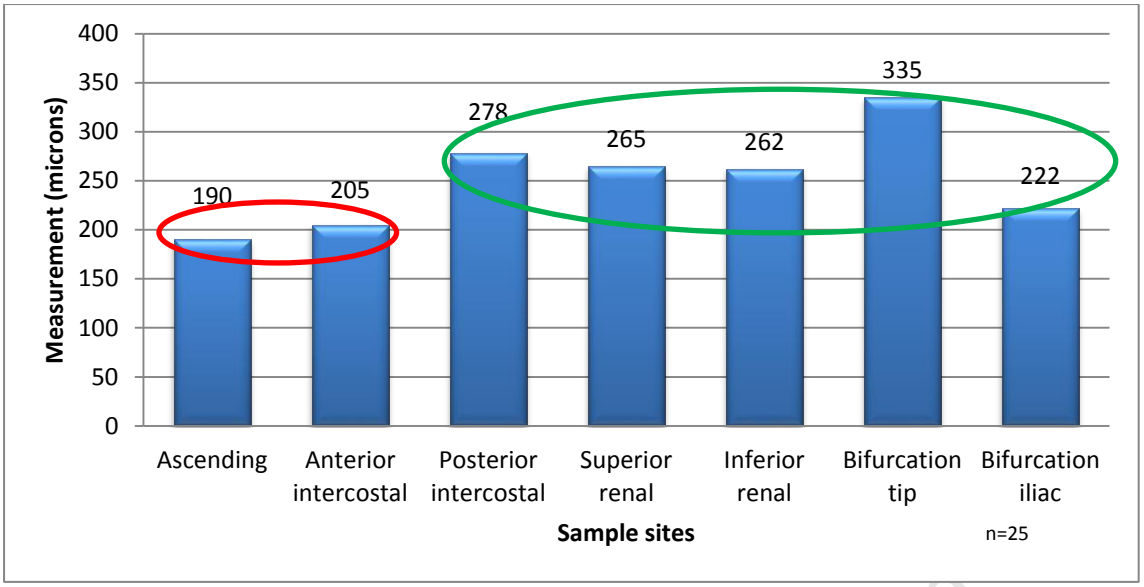

Figure 4.38: Average tunica intima thickness at branching and non-branching sites along the aorta

Note red ring indicating non-branching sites and green ring indicating branching sites

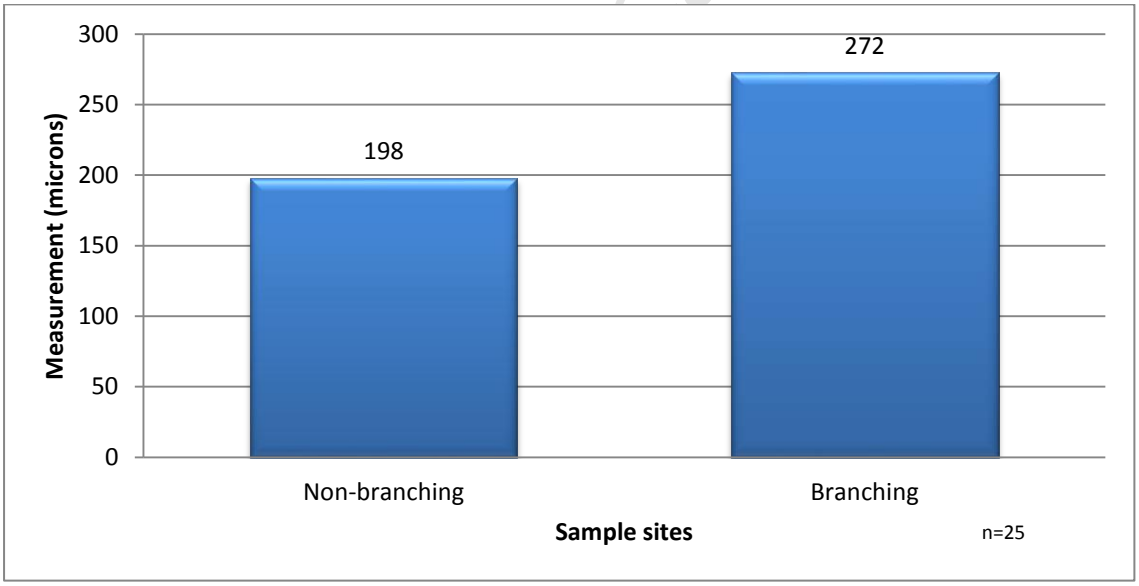

Figure 4.39: Combined average tunica intima measurements at branching and non-branching sites along the aorta

The average tunica media measurements at branching and non-branching sites are illustrated in Figure 4.40 and 4.41 below. With the exception of the average tunica media thickness at the bifurcation tip site, the average tunica media measurements of the non-branching sites are larger than those of the branching sites. Results from Figure 4.41 show that the average

tunica media measure for the non-branching sites is 1 234microns, while the average tunica media measure for the branching sites is 1 072 microns. There was a statistically significant difference between the average tunica media measurements at the branching and non-branching sites (t=-3.97, p=0.0001).

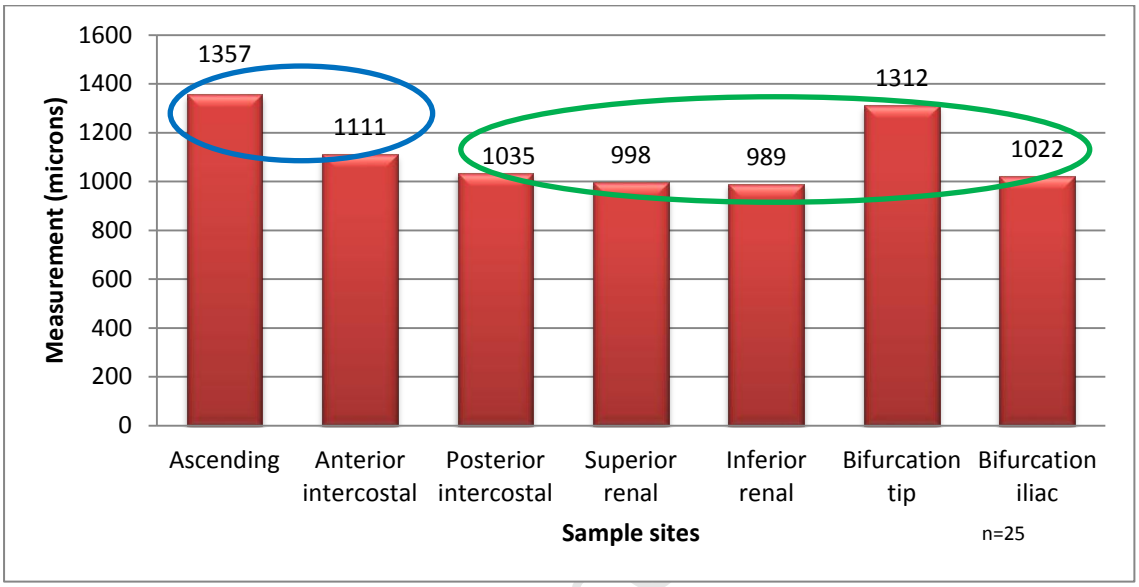

Figure 4.40: Average tunica media thickness at branching and non-branching sites along the aorta

Note blue ring indicating non-branching sites and green ring indicating branching sites

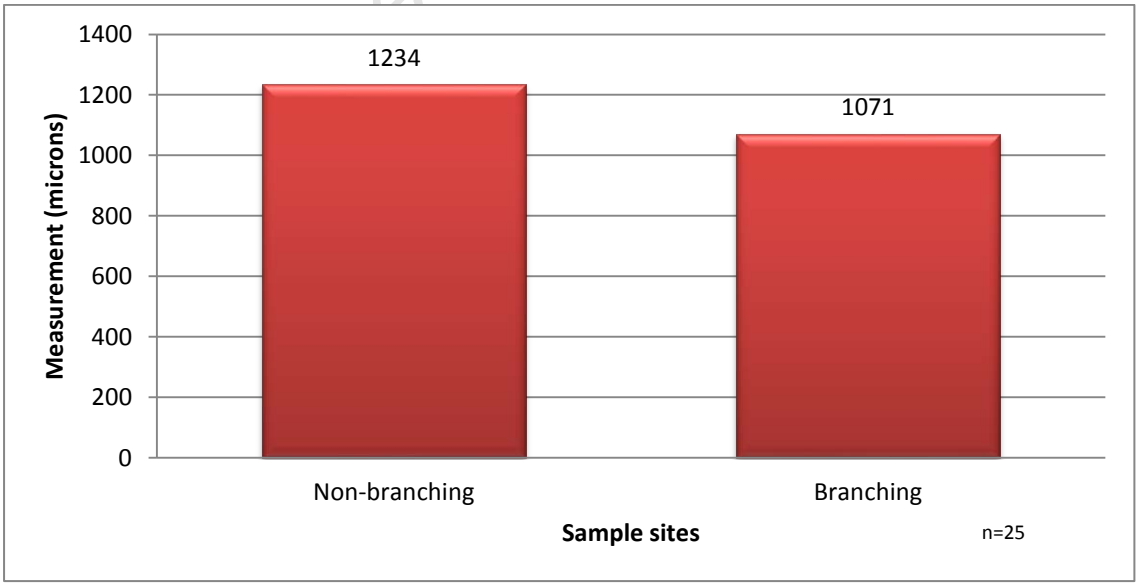

Figure 4.41: Combined average tunica media measurements at branching and non-branching sites along the aorta

Examples of different thicknesses of the tunica intima and the tunica media

The two micrographs below (Figure 4.42 and Figure 4.43) are examples of the sections analysed and illustrate the different thicknesses of the tunica intima and the tunica media in at different sites of the same individual. The first micrograph (Figure 4.42) is a section taken at the ascending site. Note the relatively thin tunica intima and thicker tunica media.

The second micrograph (Figure 4.43) was taken from the superior renal site and viewed at the same magnification in the same aorta as that shown in the first micrograph (Figure 4.42). Note the increased thickness of the tunica intima and the reduced thickness of the tunica media.

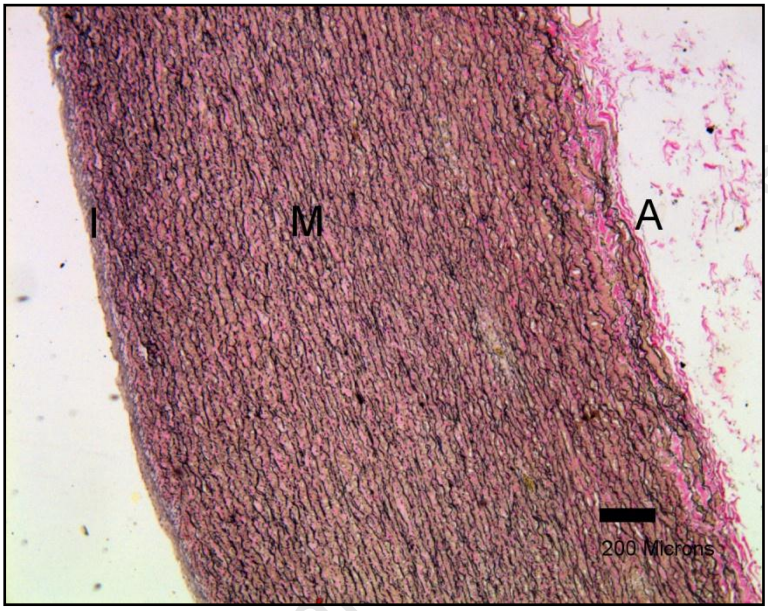

Figure 4.42: Micrograph of a vessel wall indicating tunica diameters of a section taken from the ascending aorta in a 26 year old Black female (EVG stain)

I: Tunica intima, M: Tunica media, A: Tunica adventitia

University of the Western Cape

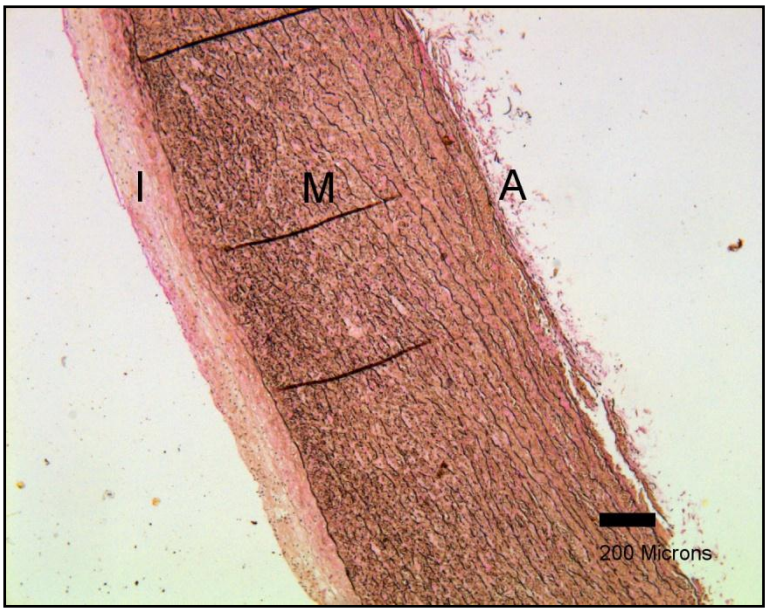

Figure 4.43: Micrograph of a vessel wall indicating tunica diameters of a section taken from the superior renal site of the descending aorta in a 26 year old Black female (EVG stain)
I: Tunica intima, M: Tunica media, A: Tunica adventitia

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4.3.1.3 Intimomedial ratio

Average intimomedial ratios at sample sites

The intimomedial ratio at each of the 7 sites in all 25 samples was calculated. As with the average tunica measurements, the intimomedial ratios for each site were averaged (Figure 4.44). The largest average intimomedial ratio was 0.30, at the posterior intercostal artery site, while the smallest average intimomedial ratio was 0.08, at the ascending sample site.

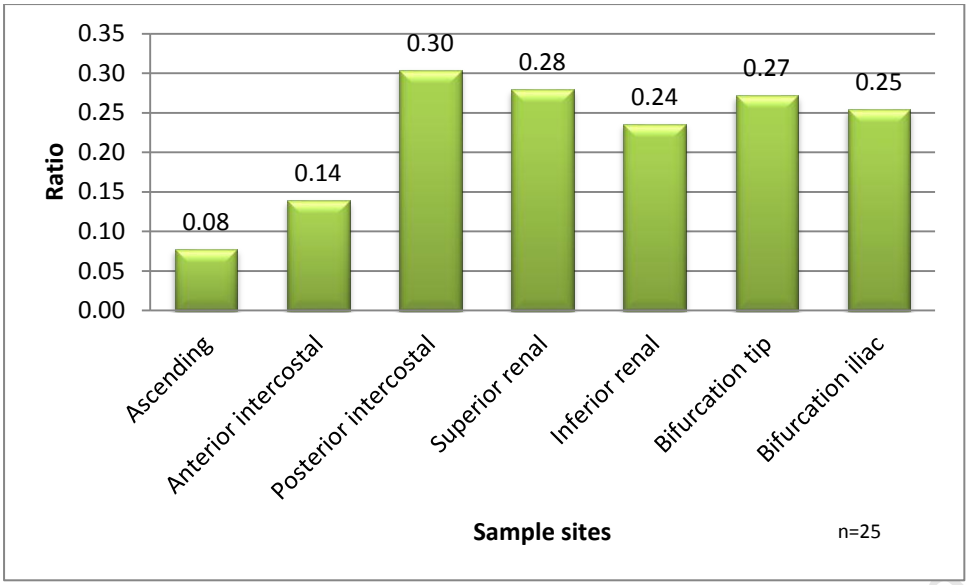

Figure 4.44: Average intimomedial ratios for each of the 7 sites along the aorta

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Comparison of branching and non-branching sites

The average intimomedial ratios of the branching and non-branching sites were compared. Figures 4.45 and 4.46 illustrate the difference between these average ratios. The average intimomedial value for the non-branching sites was 0.11, while that of the branching sites was 0.27. There was a statistically significant difference between the average intimomedial ratios at the branching and non-branching sites (t=3.97, p=0.0001).

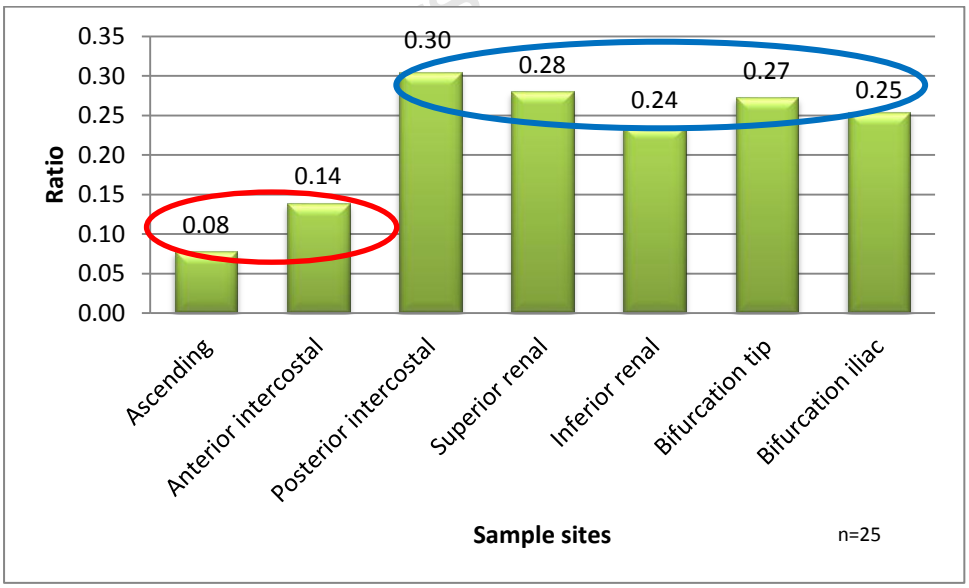

Figure 4.45: Average intimomedial ratios at non-branching and branching sites along the aorta

Note red ring indicating non-branching sites and blue ring indicating the branching sites

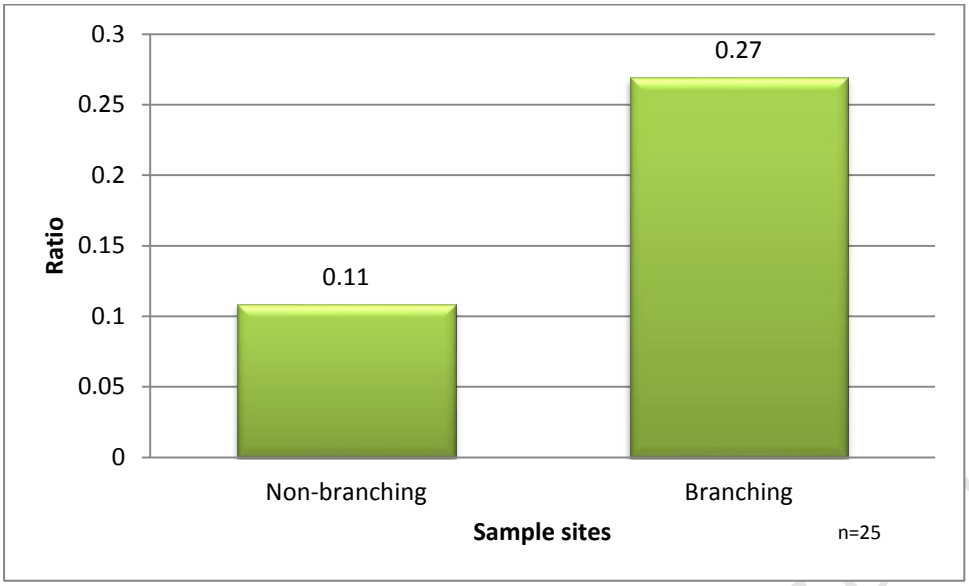

Figure 4.46: Combined average intimomedial ratios at non-branching and branching sites along the aorta

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4.3.1.4 Elastin fragmentation and grading

Average elastin fragmentation grading for sample sites

Elastin fragmentation was measured and graded for each of the 25 samples, based on the elastin fragmentation grading system proposed by Schlatmann & Becker (1977). The average elastin grade for each sample site was then calculated in order to compare levels of elastin fragmentation between the sample sites along the aorta. Figure 4.47 illustrates the average elastin fragmentation grade for each site. The least amount of elastin fragmentation was observed at the anterior intercostal site, with an average grade of 1.17. The greatest amount of elastin fragmentation was observed at the bifurcation sites, with an average grade of 2.96 at the bifurcation tip and 2.92 at the bifurcation iliac sites.

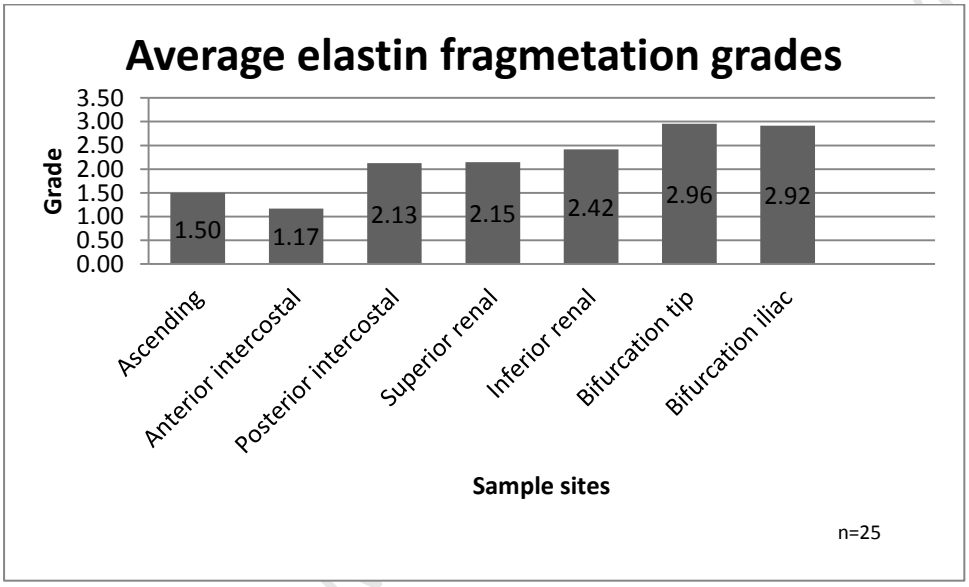

Figure 4.47: Average grade of elastin fragmentation for each site along the aorta

Comparison of branching and non-branching sites

The elastin fragmentation at both non-branching and branching sites was assessed. Average elastin fragmentation grades for the non-branching and branching sites were calculated and compared. The results, as illustrated in Figure 4.48 and 4.49, indicate that the average elastin fragmentation for the non-branching sites was 1.33, while that of the branching sites was 2.51. This indicates that the elastin fragmentation at branching sites was worse than that at non-branching sites, as seen in Figures 4.50, 4.51 and 4.52. The difference between the average elastin fragmentation grades at the branching and non-branching sites was statistically significant (t=12.45, p=0.00).

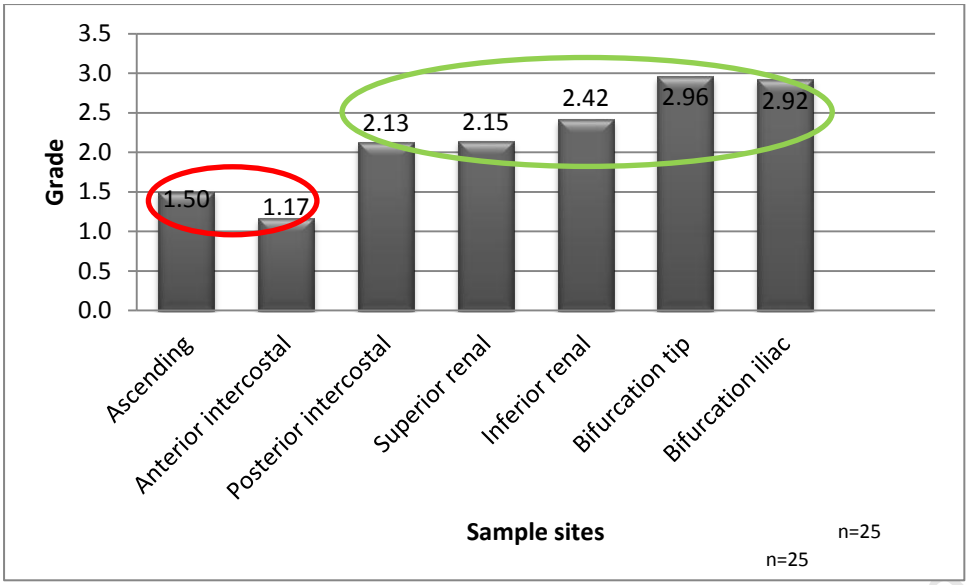

Figure 4.48: Average elastin grades for non-branching and branching sites along the aorta Note red ring indicating non-branching site and green ring indicating branching sites

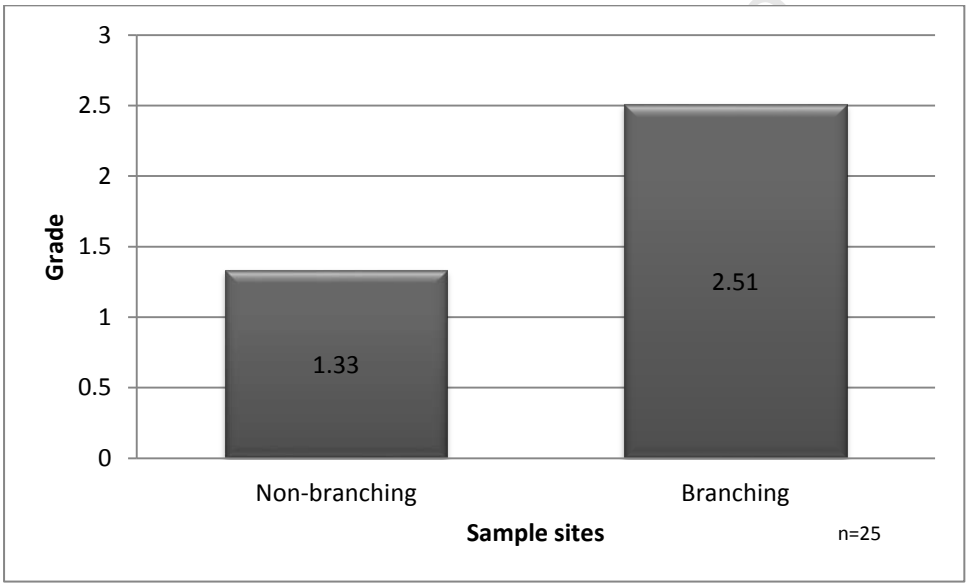

Figure 4.49: Combined average elastin grades for non-branching and branching sites along the aorta

Examples of elastin fragmentation

The three micrographs below are examples of sections taken from the same individual and illustrate the difference in elastin fragmentation at non-branching and branching sites. The first micrograph is a section taken at the anterior intercostal site (Figure 4.50). Note the abundance of elastin fibres (black lines) in the tunica media.

The second micrograph was taken from the inferior renal site (Figure 4.51). Note the increase in elastin fragmentation in the tunica media.

The third micrograph was taken from the bifurcation tip site (Figure 4.52). Note the extent of elastin fragmentation in the tunica media.

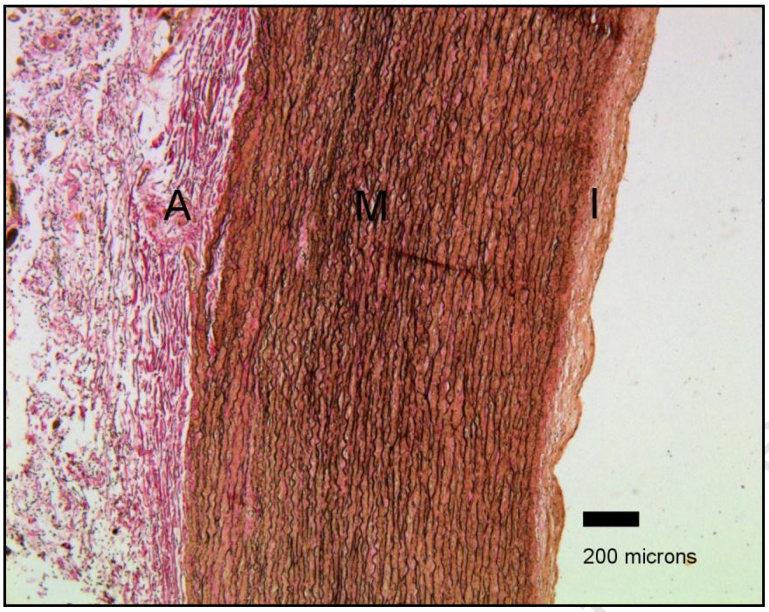

Figure 4.50: Micrograph indicating minimal (Grade 1) elastin fragmentation in a section taken from the non-branching, anterior intercostal site of a 33 year old Black female (EVG stain)

I: Tunica intima, M: Tunica media, A: Tunica adventitia

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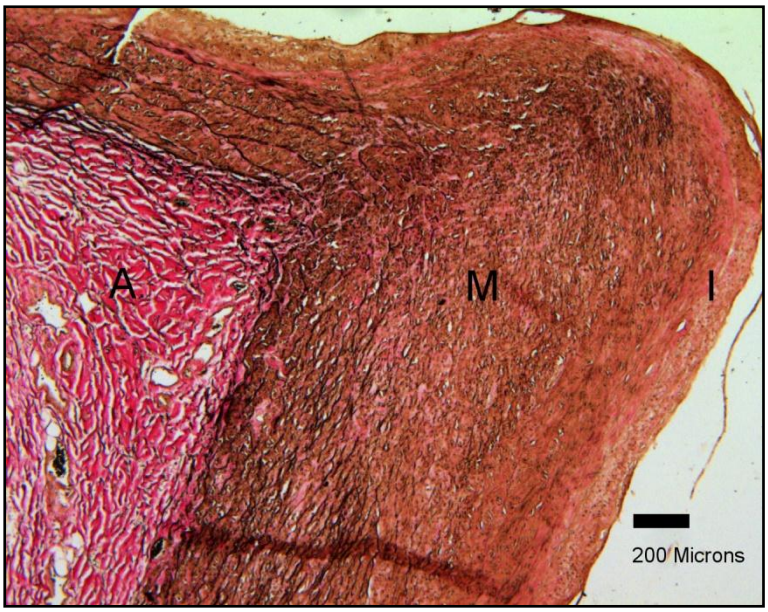

Figure 4.51: Micrograph indicating Grade 2 elastin fragmentation in a section taken from the branching, inferior renal site of a 33 year old Black female (EVG stain)

I: Tunica intima, M: Tunica media, A: Tunica adventitia

Note severe elastin fragmentation on intimal side of the tunica media

of Cape Town

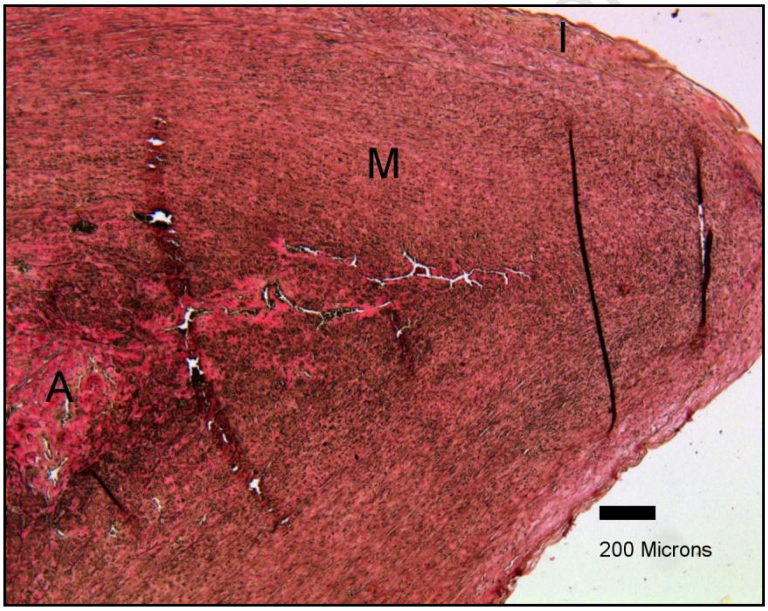

Figure 4.52: Micrograph indicating Grade 3 elastin fragmentation in a section taken from the branching, bifurcation tip site of a 33 year old Black female (EVG stain)

I: Tunica intima, M: Tunica media, A: Tunica adventitia

4.3.2 Histology of the aortic ridge

The histological features of the aortic ridge were investigated in 10 of the 149 aortic ridge samples collected from the Salt River Mortuary.

4.3.2.1 Acid mucopolysaccharides

The presence of abundant acid mucopolysaccharides is evident in the example illustrated in Figure 4.53. All samples examined were positive for acid mucopolysaccharides.

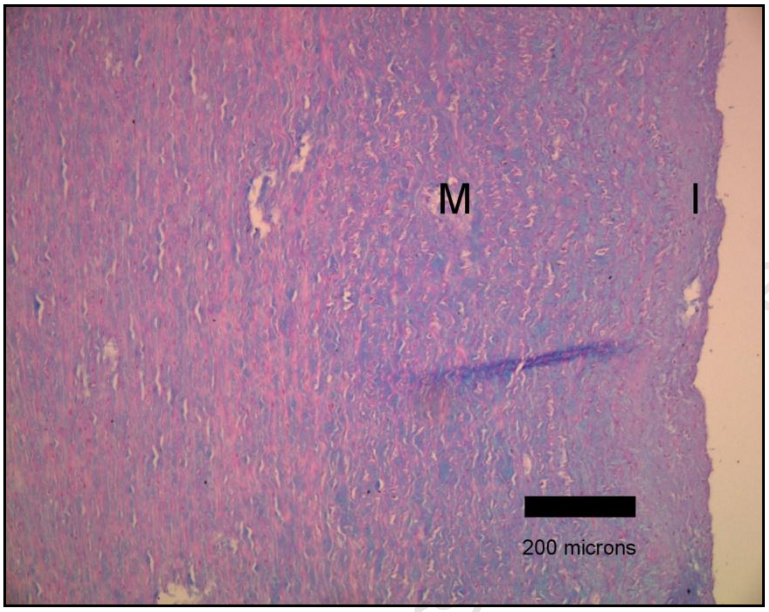

Figure 4.53: Micrograph indicating acid mucopolysaccharides (blue) in the ascending aorta of a 35 year old Coloured male (AB-PAS stain)
I: Tunica intima, M: Tunica media

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4.3.2.2 Vessel wall measurements

Average tunica intima thickness

Average tunica intima thickness for the ridged and non-ridged sample sites were compared (Figure 4.45). The average tunica intima thickness for the non-ridged site was 137 μ, and that of the ridged samples were 132 μ. No significant difference between the average tunica intima thickness of the non-ridged and ridged samples were noted (t=-0.21, p=0.84).

Average tunica media thickness

Comparisons of the average tunica media thickness for the ridged and non-ridged sample sites were made. The average tunica media thickness for the non-ridged site was 1261 μ, compared to 1885 μ for the ridged site (Figure 4.54). There was a significant difference between these two values (t=3.35, p=0.002).

Average tunica thickness

Figure 4.54 shows both the average tunica intima and media thickness for the ridged and non-ridged sample sites. The results from this graph indicate the difference between the tunica media thickness was greater than the difference between the tunica intima thicknesses for the two samples.

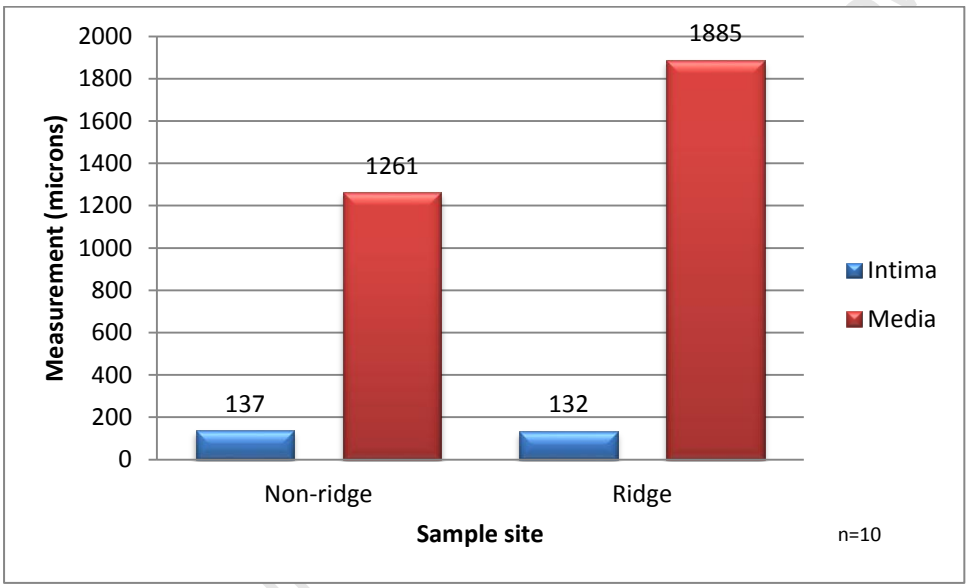

Figure 4.54: Average tunica thickness (μ) at each sample site in the aortic ridge sample

Examples of different thicknesses of the tunica intima and the tunica media

Figure 4.55 and Figure 4.56 below are examples of the sections analysed and illustrate the different thickness of the tunica intima and the tunica media. The first micrograph (Figure 4.55) is a section taken at the non-ridged site.

The second micrograph (Figure 4.56) was taken from the ridged site in the same aorta shown in the first micrograph. Note the increased thickness of the tunica media.

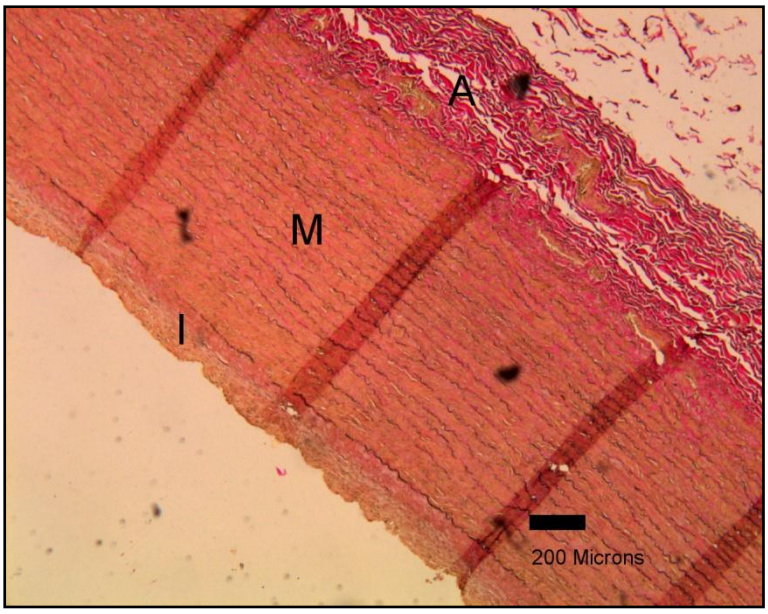

Figure 4.55: Micrograph of a vessel wall indicating tunica thickness of a section taken from the non-ridged site in a 22 year old Black male (EVG stain)
I: Tunica intima, M: Tunica media, A: Tunica adventitia

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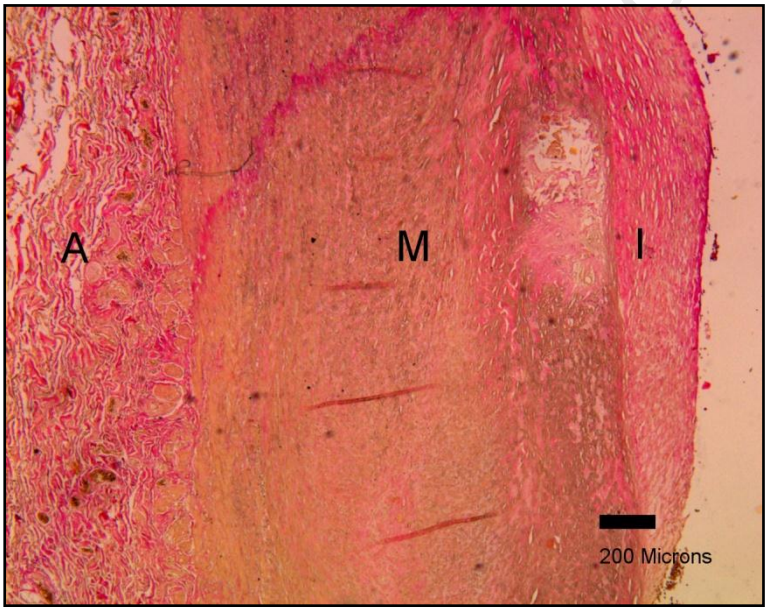

Figure 4.56: Micrograph of a vessel wall indicating tunica thickness of a section taken from the ridged site in a 22 year old Black male (EVG stain)
I: Tunica intima, M: Tunica media, A: Tunica adventitia

4.3.2.3 Intimomedial ratio

The intimomedial ratio at each site was calculated. As with the average tunica thickness, the intimomedial ratios for each site of the 25 samples were averaged to form an average intimomedial ratio for each sample site. The average intimomedial ratio at the non-ridged site was 0.11, while the average intimomedial ratio at the ridged site was 0.07. There was no significant difference between the intimomedial values at the non-ridged and ridged sites (t=-0.81, p=0.43).

4.3.2.4 Elastin fragmentation

The elastin fragmentation at the ridged and non-ridged sites was assessed and graded. The average elastin grade for the non-ridged site was 1.10, while the average elastin grade of the ridged site was 2.90. There was a significant difference between these two values (t=12.73, p=0.00).

Examples of elastin fragmentation

The two micrographs below are examples of sections taken from the same individual and illustrate the difference in elastin fragmentation at ridged and non-ridged sites. Figure 4.57 below is a micrograph indicating a section taken from the non-ridged site. Note the abundance of elastin fibres (black lines) in the tunica media.

Figure 4.58 is the second micrograph below and was taken from the ridged site. Note the extent of elastin fragmentation in the tunica media.

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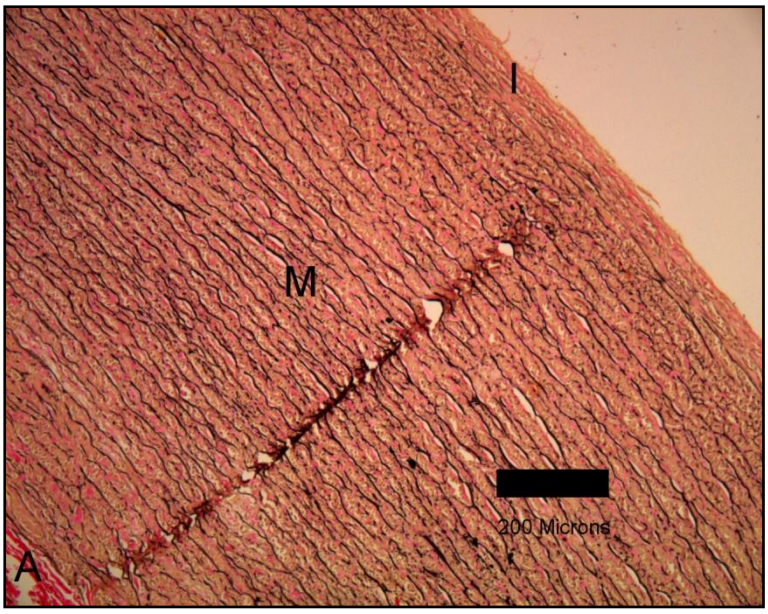

Figure 4.57: Micrograph indicating minimal (Grade 1) elastin fragmentation in a section taken from the non-ridged site of a 23 year old White female (EVG)
I: Tunica intima, M: Tunica media, A: Tunica adventitia

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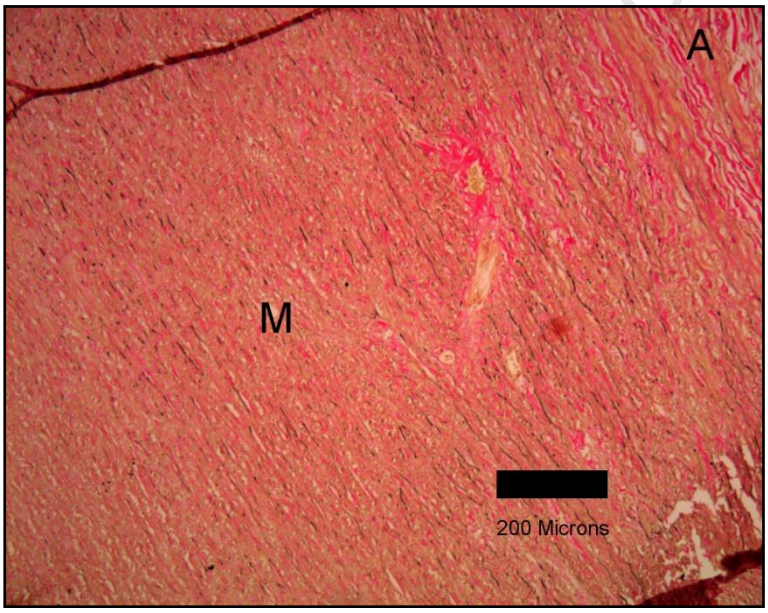

Figure 4.58: Micrograph indicating Grade 3 elastin fragmentation in a section taken from the ridged site of a 23 year old White female (EVG)
M: Tunica media, A: Tunica adventitia
Note lack of elastin tissue

Chapter 5: Discussion

5.1 Introduction

The aims of this study were to investigate the variation in the branching pattern of the aorta and to document the histological structure of this major blood vessel. The results of this investigation indicate that variations in branching patterns of the aorta were found frequently in the cadaver sample. These included some exceptionally rare branching patterns.
Variation of the histological structure of the aorta in the mortuary sample was observed. Most noteworthy was the abundance of acid mucopolysaccharides and an increase in the tunica intima thickness and elastin fragmentation of the tunica media at branching sites along the aorta.
Lastly, the identification of an aortic ridge, as observed in the majority of the mortuary sample, was noteworthy. This ridge, found on the luminal surface of the aorta at the junction of the aortic arch and descending aorta, to the knowledge of the authors of this study, has not been documented in humans and published in the literature before.

This chapter will start by discussing the results of the gross anatomy followed by the histological variation of the aorta. An introduction on aortic ridges is presented, followed by a discussion on the prevalence and histological structure of the aortic ridge. Finally, the limitations of the study will be addressed.

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5.2 Sample demographics

5.2.1 Cadaver sample

Almost three quarters (73%) of the cadaver population was male as compared to 48% in the general South African population (Statistics South Africa, 2011). The increased number of males in the cadaver population may be due to 28% of the cadavers were individuals that were unclaimed from state hospitals after death. All of these unclaimed bodies, except for one, were male. Goy (1992) suggests that female paupers are more likely to be claimed from state hospitals than male paupers. This may be an explanation for the majority of unclaimed bodies being male.

The cadaver population is also skewed in terms of race. The majority (79%) of the South African population are Black individuals (Statistics South Africa, 2011). The majority (59%) of the cadaver population in the present study are White individuals (59% vs. 9% in the South African population). The large number of White cadavers may be a result of cultural beliefs among the different racial groups in the South African population.

The majority of the cadavers used in this study were donations or bequests and all those, except two, were White individuals. Cultural belief is a factor that emerged in a study conducted by DeJong et al. (as cited in West & Burr, 2002). In this study it was found that cultural beliefs concerning organ donation and transplantation differed significantly between donor and non-donor respondents. These findings are confirmed by Kometsi & Louw (1999) in which Black South African families' main concerned was that donating an organ would mean the deceased would become a "complaining ancestor". The findings in these studies provide an insight into the cultural beliefs and how beliefs can influence decisions in body donations particularly those in Black South Africans. Hence, the cultural beliefs among the different racial groups in the cadaver sample may explain the increased numbers of White individuals.

The observation that a large proportion (72%) of the cadaver population is older than 80 years may be, once again, due to the fact that 72% of the cadavers were bequeathed. A possible explanation for this may be that older people are more likely to contemplate death and dying. If this were the case it makes sense that elderly people are more likely to consider donating their bodies to science and therefore the percentage of bequests is higher amongst them. There might also be a sense of "wanting to make a difference" in those who bequeath

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their bodies and thus they might feel that by donating their body they are making a difference.

The discussion and conclusions drawn from the results in this study may not be representative of the general South African population. The cadaver population is less representative of the South African population as the majority of these individuals are White elderly males.

5.2.2 Mortuary sample

The mortuary sample compared to the cadaver sample is more representative of the South African population as the majority of the mortuary samples were Black individuals (55%) with an average age of 37.1 years. However, the majority were still male (82%) with a large population of Coloured individuals (30%). Therefore, the discussion and conclusions drawn from the results regarding the mortuary population may not be representative of the general South African population either.

The majority of the mortuary samples were male and a possible explanation for this observation is that these samples were collected from Salt River Mortuary. These samples are therefore from individuals involved in forensic cases, the majority of which are homicide cases involving young male individuals.

The increased percentage of Coloured individuals (9% in the South African population compared to 30% in the histology sample) may be explained by the catchment area of the Salt River Mortuary, i.e. forensic cases from the greater Cape Town region. This region has a greater Coloured population (54%) compared to South Africa as a whole (Statistics South Africa, 2011).

The majority of the mortuary sample (57%) is between the ages of 20 and 39 years. Once again, this may be due to the majority of these individuals being involved in forensic homicide cases.

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5.3 Variation in the branching pattern of the aorta

The frequency of variation along the length of the aorta in the cadaver sample was found to be 49%. Note that there was no association found between presence of variation in branching patterns and sex or between presence of variation in branching patterns and race of the individuals. For the purpose of this discussion, the patterns of variation will be divided into aortic arch branching variation and branching variation present along the descending aorta.

5.3.1 Variation in the branching pattern of the aortic arch

Knowledge of variations in branching patterns along the aortic arch is useful to anatomists, radiologists and head, neck, thoracic and vascular surgeons. Many of these variations are described and discussed in the literature. The prevalence of the variation in this study is compared to other studies and also discussed in terms of their clinical significance.

Total variation in branching patterns of the aortic arch in this study differed, in some instances considerably, from that published in the literature. The most common branching pattern of the aortic arch consists of 3 branches: the brachiocephalic trunk, the left common carotid artery, and the left subclavian artery. This pattern was observed in 65% of the cadaver sample. This is much lower than the normal frequency of this branching pattern described elsewhere. One such study, conducted by Nelson & Sparks (2001), found the branching patterns described in as many as 94% of their sample (mortuary samples from men of Japanese ancestry born in Hawaii). This indicates increased frequency of variation found in the current sample.

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5.3.1.1 Left vertebral artery

In most cases (Standring et al., 2006) the vertebral arteries arise from the superior posterior aspect of the first part of the subclavian arteries. They pass through the transverse processes of the C6-C1 vertebrae. The right and left vertebral arteries enter the cranium via the foramen magnum and fuse to become the basilar artery on the ventral aspect of the pons. They thus supply blood to the upper spinal cord, the brain stem, cerebellum, and occipital lobe of the cerebrum.

Presence of a left vertebral artery arising as a separate branch off the aortic arch was observed in 6 of the 71 cadavers or 8.5%. This frequency is higher than that described in the literature (Figure 5.1). Nizanowski et al. (1982) reported a frequency of 1.5% of this branching pattern in their sample, Daseler & Anson (1959) report a frequency of 1-2.5% in their sample, whilst Voster et al. (1998) have found this pattern in as many as 5 %. Interestingly, the only other study with a frequency of variation above 2.5% was that of Voster et al. (1998) which happen to be the only other South African study found in the literature. This possibly indicates that South African cadaver populations have an increased incidence of this type of variation.

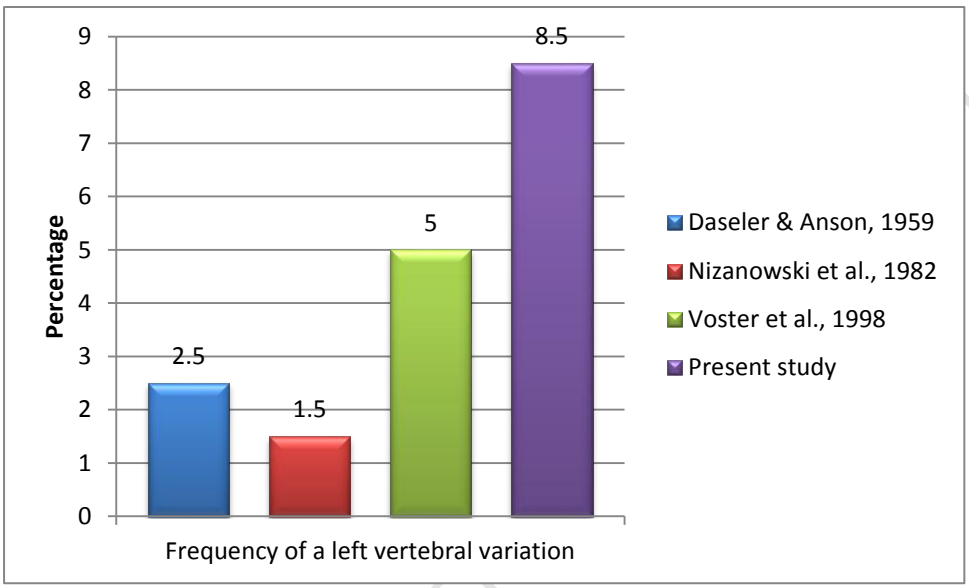

Figure 5.1: Comparison of the frequencies of left vertebral variation of the aortic arch in different studies

Patients with variations of the left vertebral artery are described to be asymptomatic in the literature. However the clinical significance of knowing the frequency and description of this variation is in the planning of aortic arch surgery and endovascular interventions (Goray et al., 2005).

5.3.1.2 Common brachiocephalic trunk

A common brachiocephalic trunk is described as the brachiocephalic trunk and the left common carotid artery branching off the aortic arch as one common trunk. Thus the aortic

arch has two branches; the common brachiocephalic trunk and the left subclavian artery (Standring et al., 2006).

The brachiocephalic trunk is the largest artery to branch off the aortic arch and has two terminal branches, the right common carotid and right subclavian artery. Occasionally a thyroidea ima, thymic or bronchial branch may arise from the brachiocephalic trunk (Moore et al., 2010).

A common brachiocephalic trunk was the most frequent variation observed in the present study and in the published literature. The current study observed this variation in 22.5% of the cadaver sample. When comparing this frequency to those published in the literature, it is a great deal higher (Figure 5.2). Nelson & Sparks (2001), report a frequency of 1%, Moskowitz & Topaz (2003) report 3.2%, and Adachi (1928), reports 11%.

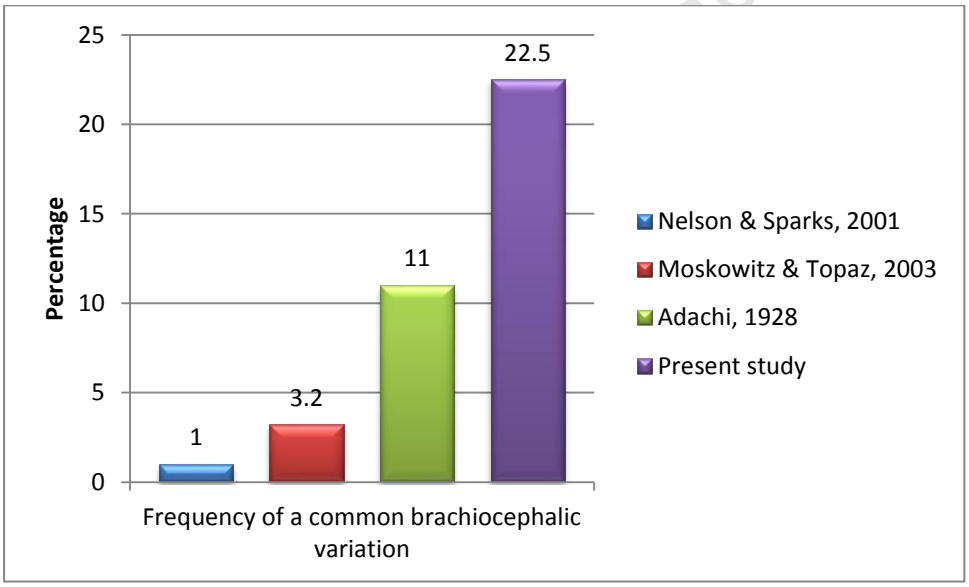

Figure 5.2: Comparison of the frequencies of a common brachiocephalic variation of the aortic arch in different studies

If the common brachiocephalic trunk is obstructed, it can pose a significant threat in terms of cerebrovascular accident as the trunk supplies three of the four sources of cerebral blood flow (Azakie et al., 1999). Although the obstructions of this vessel from occlusive atherosclerosis may occur, such occurrences have been addressed by surgical interventions. Revascularization of a common brachiocephalic trunk has been achieved using either a

prosthetic bypass graft or endarterectomy (Azakie et al., 1999). The high frequency of this variation in the present cadaver sample and the potential for this pattern to pose a significant threat in terms of cerebrovascular accident, does, however need to be taken into account.

5.3.1.3 Right and left brachiocephalic trunk

The rare variation of a right and left common brachiocephalic trunk has been reported to occur in 1.2% of sample populations (Anson, 1963, as cited in Paraskevas et al., 2008). However, this variation is not well documented in the literature and no significant clinical implication has been reported. The frequency of 1.4% observed in the current study is very similar to that of 1.2% observed by Anson (1963, as cited in Paraskevas et al., 2008).

5.3.1.4 Retro-oesophageal right subclavian artery

The right subclavian artery is usually a branch of the brachiocephalic trunk. It supplies blood to the brain and upper spinal cord via its vertebral branch, and supplies the shoulder and upper thorax via its internal thoracic, thryrocervical, costocervical, and dorsal scapular branches. It continues into the axilla and upper limb via its continuation, the axillary artery.

The presence of a retro-oesophageal right subclavian artery is rare and only presents in 0.4-2% of cadaver and radiographic samples (Bergman et al., 1988). The observation of a single case in the present sample of 71 cadavers is consistent with the reported frequency of occurrence and confirms the rarity of this variation.

Numerous case reports of retro-oesophageal right subclavian arteries have been published due to the clinical significance. Difficulty in swallowing, D*ysphagia lusoria* in adults may be caused by a retro-oesophageal right subclavian artery when advanced atherosclerosis and hardening of the vessel causes compression of the oesophagus (Beauchamp et al. 1978).

Abnormal aortic arch development, such as retro-oesophageal right subclavian artery development, causes variation in the course of the right inferior laryngeal nerve. Usually the right recurrent laryngeal branch of the vagus nerve descends into the thoracic cavity and loops around the right subclavian artery, before rising up into the neck to supply motor and sensory innervations to the larynx. However, with a retro-oesophageal right subclavian

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artery, the right inferior laryngeal nerve may branch directly from the vagus nerve without descending into the thoracic cavity, i.e. a non-recurrent inferior laryngeal nerve.

Non-recurrent inferior laryngeal nerve variations have been described by Vuillard et al. (1978) and by Uludag et al. (2009). In the study by Uludag et al. (2009), a right non-recurrent laryngeal nerve was associated with a right subclavian artery branching off the aortic arch, whereas Vuillard et al. (1978) have described numerous types of non-recurrent inferior laryngeal nerve variations. In the present study, the right inferior laryngeal nerve was dissected and its course noted. The right inferior laryngeal nerve branched directly from the vagus nerve and had a course describe by Vuillard et al. (1978) as "Type 1" variation of non-recurrent laryngeal nerve.

Variations in the nervous innervations of the larynx are of clinical significance, as nerve damage during surgery can easily occur if the presence of a non-recurrent inferior laryngeal nerve is unknown (Uludag et al., 2009). This is of particular concern for those individuals with the retro-oesophageal right subclavian artery in need of thyroid surgery (Avisse et al., 1998).

The embryological developmental of a retro-oesophageal right subclavian artery anomaly has been described to occurs when degeneration of the fourth vascular arch, along with the dorsal aorta, leaves the seventh intersegmental artery attached to the descending aorta (Abhaichand et al., 2001). The persistent seventh intersegmental artery then assumes a retro-oesophageal or alternatively retrotracheal position as it proceeds out of the thorax into the right upper limb to become a retro-oesophageal right subclavian artery or a reto-trachial right subclavian artery.

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5.3.1.5 Common brachiocephalic trunk and a common trunk of the left vertebral and left subclavian arteries

Only one other case with a similar, but not identical, variation could be found in the literature (Çetin et al., 2009). No clinical significance is noted in the literature, but the knowledge of such a variation would be relevant and useful to anatomists, radiologists and head, neck, thoracic and vascular surgeons.

5.3.2 Variation in the branching pattern of the descending aorta

5.3.2.1 Supernumerary posterior intercostal arteries

The first two pairs of posterior intercostal arteries are described as originating from the supreme intercostal artery, a branch of the costocervical trunk. The remaining nine pairs (3rd to 11th) arise from the dorsal aspect of the descending aorta. The nine posterior intercostal arteries supply blood to the lower nine intercostal spaces. Each of these arteries anastomose with a corresponding anterior intercostal artery. The lower two arteries however, anastomose with the subcostal, superior epigastric and lumbar arteries in the anterior abdominal wall (Standring et al., 2006).

A high incidence of variation in the origin of the posterior intercostal arteries was reported by Khan and Haust (1979). There was no clinical significance given to these findings and subsequent searching of the literature didn't reveal any significance relating to the variation in the origin of these vessels. The frequency of variation in the current study (6%) is less than that reported by Khan and Haust (1979). Khan and Haust (1979) found the presence of a single supernumerary artery in 55 instances, and supernumerary of a pair of arteries in 14 cases of a total of 79 samples (17.7%).

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5.3.2.2 Multiple renal arteries

The paired renal arteries arise from the lateral aspects of the aorta, just below the origin of the superior mesenteric artery. The right renal artery is typically longer and often branches off slightly more proximally along the aorta than the left renal artery. The renal arteries deliver approximately 20% of total cardiac output to supply the kidneys which represent less than one-hundredth of the total body weight (Standring et al., 2006) in order to excrete the end products of metabolism and excess water.

Frequencies of multiple renal arteries have been recorded in as many as 30% of individuals and occur predominantly on the left hand side (Williams et al., 1995). However, Janschek et al. (2004), observe multiple renal arteries in 20.2% on the right hand side and 19% on the left side of individuals. These frequencies are higher than those observed in this study (28% total; 14% on both right and left hand sides).

The knowledge of multiple renal arteries is useful and relevant to academics, surgeons and radiologists due to the increased number of laparoscopic surgeries and renal transplantations taking place (Das, 2008). Multiple arteries have been described to pass to the lower pole of the kidney more often than to the upper pole of the kidney (Graves, 1969). This is clinically important as the accessory vessels to the inferior pole often cross anterior to the ureter, which may cause obstruction of the ureter leading to hydronephrosis (Singh & bay, 1998).

5.3.2.3 Coeliacomesenteric artery

The coeliac trunk or axis is the first unpaired branch of the abdominal aorta and arises at the level T12/L1 vertebral bodies. The coeliac trunk divides into the left gastric, common hepatic and splenic arteries, which supply the lower oesophagus, stomach, liver, spleen and portions of the duodenum and pancreas.

The superior mesenteric artery arises inferior to the coeliac trunk at the level of the L1-2 intervertebral disk. The superior mesenteric artery has numerous branches; the inferior pancreaticoduodenal artery, the middle colic, the right colic, many ileal and jejunal arteries, and the ileocolic artery. The pancreaticoduodenal artery supplies blood to the head of the pancreas and the lower portion of the duodenum. The middle colic artery supplies blood to two thirds of the transverse colon. The jejunal arteries branch from the left side of the upper portion of the superior mesenteric artery to supply the jejunum with blood, while the ileal arteries branch from the left and anterior portion of the superior mesenteric artery to supply blood to the ileum. The ileocolic artery supplies blood to the caecum, distal ileum and appendix (Standring et al., 2006).

The embryological development of coeliac and mesenteric arteries and the hepatic arterial vascularisation are well documented (Douard et al., 2006,). The incidence of a common coeliacomesenteric artery of 3% in the current study falls into the range of 2-5% observed by Rio Branco da Silva, (1912), Lippert & Pabst (1985), Vandamme & Bonte, (1990), Douard et al. (2006). No clinical significance for this variation was found in the literature; however knowledge of its frequency of occurrence may be useful to anatomists, radiologist and vascular surgeons.

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5.4 Histological variation

5.4.1 Histological variation of the aorta

5.4.1.1 Acid mucopolysaccharides

The high prevalence of acid mucopolysaccharides in the sample is noteworthy. Mucopolysaccharides are glycosaminoglycans which form the basis of ground substance. Ground substance is the medium for passage of molecules throughout supporting tissues. Glycosaminoglycans comprise of long unbranched polysaccharide chains consisting of repeating disaccharide units (Young et al., 2007) and are a constituent of the connective tissue in blood vessels.

Presence of acid mucopolysaccharides is known to be associated with age-related elastic fragmentation and increased collagen within the tunica media (Zugibe, 1962; Johnson & Burns, 1993). However, due to the young age (average age of 33 years) of the individuals from which the histological samples were taken, and no association of presence of acid mucopolysaccharides with age, age-related changes to the vessel is an unlikely explanation for the high prevalence of acid mucopolysaccharides observed.

Intimomedial mucoid degeneration is a rare and poorly understood vascular disease affecting the intima and media of aortic and extra aortic vessels causing aneurysms (Katz et al., 2008). This disease has been observed predominantly in the young Black population within South Africa with hypertension (Decker et al., 1977; Abdool-Carrim et al., 1996). Intimomedial mucoid degeneration is characterised histologically by the diffuse elastic tissue degeneration with large quantities of acid mucopolysaccharide deposition within the tunica intima and media (Abdool-Carrim et al., 1996). Given that intimomedial mucoid degeneration is a rare disorder, and the elastin fragmentation was not severe at every sample site (average elastin fragmentation grades were: 1.50 at the ascending, 1.17 at the anterior intercostal, 2.13 at the posterior intercostal, 2.15 at the superior renal, 2.42 at the inferior renal, 2.96 at the bifurcation tip, and 2.92 at the bifurcation iliac sites), and given that aneurysms were not detected macroscopically at post mortem, it seems unlikely that every one of the 25 individuals from which the histology samples were taken had this disorder. Unfortunately, the medical records for the individuals were not available and therefore a diagnosis of intimomedial mucoid degeneration disorder could not be confirmed amongst the sample.

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An alternative explanation for the high presence of acid mucopolysaccharides in the present study is the link between diet and mucopolysaccharides. Sandhyamani (1999) found that a diet high in carbohydrates and low in protein can result in a high presence of acid mucopolysaccharides in the vessel wall of monkeys. This study noted that the Trivandrum population in India, ate such a diet and a high number of these individuals were found to have acid mucopolysaccharides in their blood vessels.

The diet of the individuals in this study would be impossible to ascertain, but it has been documented that South Africans have a diet high in animal protein, fat and refined sugars (Steyn & Fourie, 2007; Voster, 2002). While this diet is not similar to that described by Sandhyamani (1999), the South African diet described by Steyn (2007) and Voster (2002), may have influenced the abundance of acid mucopolysaccharides in this sample. This possible explanation should be further investigated.

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5.4.1.2 Measurements of the vessel wall

The average tunica intima values represent average tunica intima thicknesses for each site along the aorta amongst the histology sample. The tunica intima of elastic arteries is normally slightly larger than 2 or 3 endothelial cell layers with a mean average thickness of 208μ in the abdominal aorta (Friedman et al., 1988). Thus the average intimal values at branching sites (272μ) in the current study are increased. This observation is supported by the average intimomedial ratio calculated at each site. The average intimomedial ratio is an indication of the relative thicknesses of the intima and media to one another at each site. The lower the ratio is, the less the thickness of the intima is compared to the thickness of the media. The average intimomedial ratio of the branching sites (0.27) was significantly higher than those of the non-branching sites (0.11). This difference in intimomedial ratios indicates there is an increased tunica intima at the branching sites along the aorta.

Schlatmann & Becker (1977) and Standring et al. (2006) have noted that the thickness of the tunica intima increases with age and is more marked in the distal than in the proximal aorta. In the present study the tunica intima was not analysed according to age as the majority of individuals were between the ages of 20 and 39 years, with only five individuals older than 40 years. Therefore no determination of correlation between thickness and age was possible.

When comparing the tunica intima thicknesses at each site with respect to the location along the aorta, there is no clear trend that indicates an intimal thickening the further distally along the aorta the sample site was located. Indeed the largest average intimal value was distal along the aorta at the bifurcation tip site (335µ), but the second largest value was at the posterior intercostal site (278µ) which is proximal along the aorta.
The average intimomedial ratios indicate that the relative thickness of the intima increased from the ascending site (0.08) to the intercostal site and peaked at the posterior intercostal site (0.30). The average intimomedial ratios were lower and remained between 0.24-0.28 for the renal and bifurcation sites. These results suggest that there is an increase in intimal thickness at branching sites compared to non-branching sites along the aorta, rather than the tunica intima thickness increasing along the length of the aorta, as proposed by Schlatmann & Becker (1977) and Standring et al. (2006).

Thus age and location along the length of the aorta (proximal vs. distal) seem unlikely reasons for the observed intimal thickening at the branching sites.

An increase in intimal thickness is indicative of atherosclerotic changes in the vessel wall (Masawa et al., 1994). These changes are characterised by intimal lesions which protrude into the lumen of the vessels and weaken the underlying media (Kumar et al., 2005). Atherosclerosis would thus seem an obvious cause for the intimal thickening. However, samples with obvious macroscopic evidence of severe atherosclerosis were not included in the histological examination of the vessel wall as atherosclerotic histological changes are well documented (Stary et al., 1992; Stary et al., 1994).
Atherogenesis is known to be an inflammatory response to endothelial injury of the arterial wall (Kumar et al., 2005) and can be caused by a number of factors which include hypertension, hyperlipidemia, smoking, immune reactions and haemodynamic forces. Among the predisposing factors leading to atherosclerosis, haemodynamical forces (shear stress and tensile stress) created by the flow of blood are of the utmost importance (Zarins et al., 1983; Asakuru & Karino, 1990; Glagov et al., 1992). These haemodynamic forces influence vessel wall structure, including tunica intima thickness, and contribute to vascular tone (Cooke et al., 1991; Glagov et al., 1993; Kuchan & Frangos, 1993; Cowan & Langille, 1996). Numerous studies (all mentioned in section 5.4.1.2) reported in the literature confirm that reduced fluid velocity, reduced shear stress and turbulent flow (flow that is not unidirectional and axially lined) are haemodynamic factors that promote atherogenesis.

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Intimal thickenings and atherosclerotic lesions tend to develop in regions where there is a separation in unidirectional laminar blood flow, which typically occurs near branches, bifurcations, regions of arterial narrowing and curvature in vessels (Zarins et al., 1983; Kim et al., 1992; Glagov et al., 1993; Davies, 1997; Carallo et al., 1999).

Thus from the evidence in the literature, the increased average tunica intima thicknesses at branching sites noted in this study are likely to be due to haemodynamic forces (reduced shear stress created by turbulent flow), rather than age and location along the length of the aorta.
Note that this is not to say that all the individuals in this study suffered from atherosclerosis, but rather physiological processes such as haemodynamic forces may cause these atherosclerotic changes such as intimal thickening early on in life. Thus haemodynamic forces appear to cause changes in vessel wall architecture in order to restore normal levels of wall shear stress. This may lead to risk factors and pathological processes compounding the problem later on in life, assisting in the further development of these changes.

The average tunica media values represent the tunica media thicknesses at each site along the aorta. No trend was observed in the thickness of the tunica media in relation to the location of the site along the aorta. Two increases in media thickness were observed. The first was at the ascending site (1357 μ), while the second was at the bifurcation tip (1312μ).
Increased tunica medial thickness at the ascending aorta site would be expected as the tunica media is comprised of smooth muscle cells, elastin and collagen which bear directly on the mechanical properties due to the increased pulsatile pressure exerted by the blood. The increase in the media thickness, as well as an increase in elastin fibres (Figure 5.3) at the ascending site may be due to these high pulsatile pressures.

The increase in tunica media thickness at the bifurcation tip site may be a result of structural adaptations due to flow separation that occurs at the bifurcation of the aorta. As blood flows down the abdominal aorta, the flow is divided into the left and right common iliac arteries at the bifurcation. It is possible that the thickened tunica media at this bifurcation site of the aorta arises as a consequence of a lack of structural support in order to maintain effective flow separation. If this were true, there would be a need for reinforcement at the bifurcation,

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particularly with collagen (Figure 5.4) for structural support, as evidenced in this study, rather than elastin for dispensability as noted in the ascending aorta.

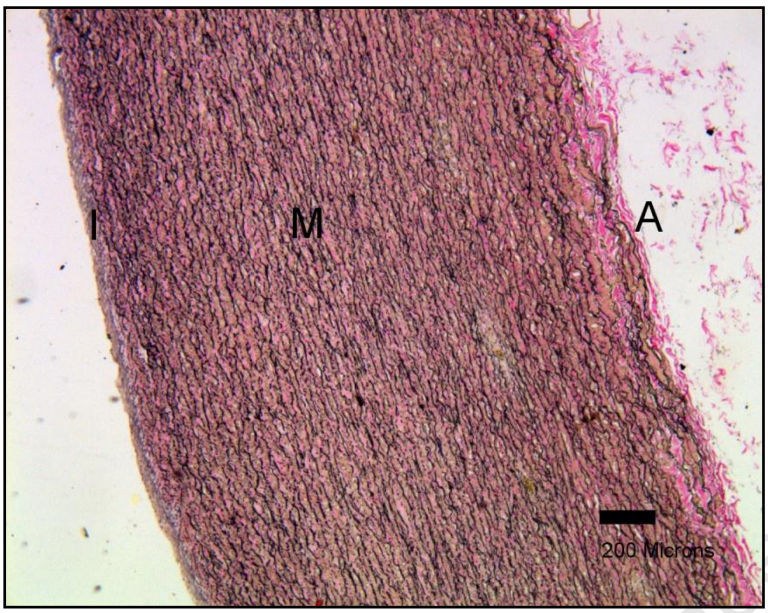

Figure 5.3: Micrograph of a vessel wall indicating increased tunica media thickness with increased elastin, from the ascending aorta in a 26 year old Black female (EVG stain)
I: Tunica intima, M: Tunica media, A: Tunica adventitia
Note abundance of elastin fibres (black) in the tunica media

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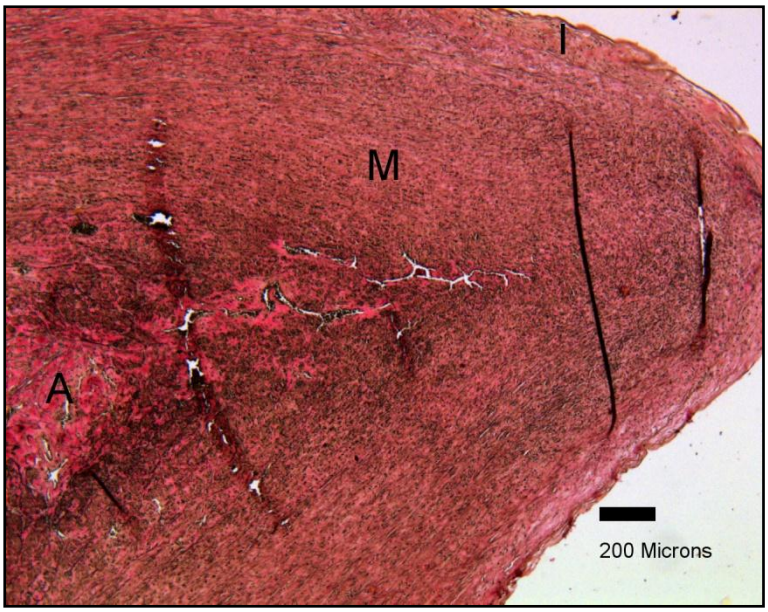

Figure 5.4: Micrograph indicating increased tunica media thickness with lack of elastin and increased collagen, from the aortic bifurcation in a 33 year old Black female (EVG)
I: Tunica intima, M: Tunica media, A: Tunica adventitia
Note abundance of collagen (pink) and lack of elastin fibres (black) in the tunica media

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5.4.1.3 Elastin fragmentation

Elastin fragmentation or disruption was observed in 91% of mortuary samples collected by Van Kets (2007). Fragmentation is characterised by the disruption of the elastin lamellae and is a common histological characteristic of the aging human aorta (Cattell et al., 1996; Schlatmann & Becker, 1977), but may also represent pathology such as annuloaortic ectasia, Marfan syndrome, HIV-positive status and tetralogy of Fallot (Halme et al., 1985; Hirata et al., 1991; Chetty et al., 2000; Tan et al., 2005).

The average grade of elastin fragmentation was calculated. The lowest grades, indicating the least elastin fragmentation, were at the anterior intercostal (1.17) and ascending sites (1.50). The highest levels of elastin fragmentation were at the bifurcation tip (2.96) and bifurcation iliac sites (2.92). This indicates that the bifurcation of the aorta has the greatest degree of elastin fragmentation. The average grade of elastin fragmentation for the branching sites was 2.51, while that of the non-branching sites was 1.33, indicating a greater degree of elastin fragmentation at the branching sites along the aorta (statistically significant; $t=12.45$, $p=0.00$).

Schlatmann & Becker (1977) describe elastin fragmentation in the normal aging aorta and discuss the implications for dissecting aortic aneurisms. Johnson et al., 2001, also describes and discusses elastin fragmentation of the tunica media of arteries with respect to age. However, age seems an unlikely reason for the elastin fragmentation seen in the present sample as very few individuals were over the age of 40 years.

The autosomal genetic disorder Marfan syndrome is known to cause abnormal elastic properties of the aorta. Patients with Marfan syndrome present with significant elastin fragmentation in segments of both the ascending and descending aorta (Hirata et al., 1991). In this study no evidence of mild or severe elastin fragmentation was observed in the ascending aorta. The distribution of elastin fragmentation from individuals in this study thus differs from that seen in those with Marfan syndrome. Elastin fragmentation was limited to branching sites along the aorta in the individuals in this study while Marfan syndrome patients have widespread fragmentation along the aorta. Therefore it is unlikely that Marfan syndrome is the reason for the elastin fragmentation observed in this study.

Congenital heart disorders have been associated with structural abnormalities of the aortic wall (Niwa et al., 2001) and included patients with tetralogy of Fallot (Tan et al., 2005) and aortic bicuspid valve replacement (Grotenhuis et al., 2007). These disorders cause elastin fragmentation in, and dilation of the aorta, which are, however, primarily only observed in the ascending aorta. The distribution of elastin fragmentation is therefore different to that seen in the present study where minimal elastin fragmentation was observed in the ascending aorta. This observation together with no sign or record of congenital heart disorder in the individuals of this sample, suggest that congenital heart disorders are not responsible for the elastin fragmentation in this study. It is presumed that individuals of this sample would have evidence identified at autopsy or a record of a congenital heart disorder if such a disorder was present.

Without a full medical record on the individuals in this sample, some possible causes for elastin fragmentation cannot be excluded. However, haemodynamic forces and HIV status are other possibilities left to be considered as responsible for the fragmentation of elastin in the vessels walls of this study. Schlatmann & Becker (1977) have previously proposed that haemodynamic forces cause this histopathological feature. In their study it was found that

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the ascending aorta was affected to a larger extent than the descending thoracic aorta. Schlatmann & Becker's (1977) findings concur with findings of the present study as the ascending site average grade of 1.50 was higher than that of the anterior intercostal site grade of 1.17. However, at the same level of the anterior intercostal site, the average grade of the posterior intercostal site was 2.13. This higher average grade indicating more elastin fragmentation at the posterior intercostal site may be due to the posterior site being a branching point. Schlatmann & Becker's (1977) did not take sections from branching points along the aorta. The present study also noted that the inner layers of the tunica media were affected more severely than the outer layers (Figure 4.59), as observed in the study by Schlatmann & Becker (1977). This observation was explained as being caused by initial haemodynamic impact within the aortic lumen and is a result of a traumatised tunica media.

Haemodynamic forces seem a logical explanation for the observed fragmentation of elastin in the tunica media within this sample as the elastin fragmentation is more severe in the ascending, compared to non-branching sites in the descending thoracic aorta where pressure and increased haemodynamic forces may cause damage to the inner layers of the tunica media. Also, branching sites in this sample had a significantly higher degree of elastin fragmentation than non-branching sites. It has been reported that branching sites or ostia are a location of altered haemodynamic forces (Zarins, et al., 1983). However, with the HIV status of the individuals of this study unknown, the exact extent to which the elastin fragmentation is caused by haemodynamic forces is yet to be determined.

Elastin fragmentation has been associated with positive HIV status (Tipping et al., 2006; Chetty et al., 2000). With an estimated 5.38 million people in South Africa living with HIV (Statistics South Africa, 2011), HIV must be considered as a potential factor for the elastin fragmentation seen in this study. As the HIV status of individuals in this study is unknown, the association between HIV status and elastin fragmentation was not investigated.

5.4.1.4 Summary of histological finding

Acid mucopolysaccharides

Acid mucopolysaccharides were abundant in the sample, occurring in all but two sections taken. A possible explanation for this observation may be the link between acid mucopolysaccharides and diet, however this needs further investigation.

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Tunica thicknesses of the vessel wall

The tunica intima at branching sites of the aorta was thickened in the sample. It is postulated that altered haemodynamic forces created by turbulent flow at branching sites is an explanation for this observation in order to restore and maintain levels of sheer stress.

An increased thickness of the tunica media was observed at the ascending and bifurcation tip sites of the aorta. The increased media of the ascending samples may be a result of high pulsatile pressure in the ascending aorta as there was an increase in elastin fibres accounting for the increased thickness of the tunica media. The increase in the thickness of the media at the bifurcation tip may be due to the increase in collagen seen in the media which might be needed in order to provide structural support at the bifurcation of the aorta to separate the flow of blood.

Elastin fragmentation

There was increased elastin fragmentation in the sample, particularly at branching sites along the aorta. Although the increased elastin fragmentation at the branching sites in this sample may be due to a combination of many of the possible causes discussed above, haemodynamic forces seem the most like cause as there is a marked difference between the elastin fragmentation at branching and non-branching sites. To what extent other factors such as HIV have an effect on the fragmentation of the elastin fibres was not examined.

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5.5 Aortic ridge

5.5.1 Introduction

An aortic ridge partially encircling the midsection of the ascending aorta on the adventitial surface has been described (Parke & Michels, 1966; Morrison et al., 2003). This well vascularised fatty ridge, situated on the right wall of the aortic bulb in opposition to the edge of the right auricle of the heart has been described as acting as a cushion (Parke & Michels, 1966). The ridge described has surgical relevance as it is well vascularised and may be a possible source of post-operative haemorrhaging.

Ridges of the luminal or intimal surface of the ascending aorta (sinutubular ridge) and left ventricle of the heart (subaortic ridge) have also been described previously in the literature (Zielinski et al., 1987; Anderson et al., 2003; Loukas et al., 2009). The ridge noted in the present study however, differs in location to those previously described as it is located on the intimal surface of the medial wall of the aorta, at the junction of the aortic arch and descending thoracic aorta.

Zielinski et al. (1987) described a subaortic ridge and observed a high prevalence of a subaortic ridge in the presence of a misaligned ventricular septal defect. Zielinski et al. (1987) speculated that there may be a common morphogenesis for misaligned ventricular septal defect, subaortic ridge, and obstructive lesions of the aortic arch.

The sinutubular junction is described as the circumferential thickening of the aortic wall at the junction between the sinus and tubular portions just above the aortic valvular cusps (Loukas et al., 2009), while a sinutubular ridge is described as the hypertrophy of this sinutubular junction (Anderson et al., 2003). Loukas et al. (2009) observed a presence of a sinutubular ridge in 62% of adult cadavers and deduced that the sinutubular ridge provides an irreversible atherosclerotic process as there is no evidence that the promoting ridge regresses. They also state that the ridge is of clinical importance particularly to those individuals who develop cardiovascular risk factors, whether genetic in nature or caused by exogenous environmental factors.

The ridge noted in the present study differs in location to those previously described and thus its description is significant.

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5.5.2 Prevalence of the aortic arch ridge

Presence of the ridge in the present study was noted in 74% of the sample, prompting further investigation to ascertain any association between sex, race and age of the individuals.

There was no statistical significant association between presence of the ridge and the sex of the individuals. Although there was a higher frequency of males present with the ridge (76% in males, 68% in females), caution should be taken when interpreting these results as there were only 19 females in the sample of 149.

Presence of the ridge was observed in 81% of the Black individuals, while only 67% of White and 66% of Coloured individuals had the ridge present. This implies that there is an association between the presence of the ridge and being a Black individual. This association between presence of the ridge and race approached significance at a 10% level and therefore there may be an association between Black individuals and the presence of the ridge. Once again caution should be taken when interpreting these results due to under representation of White and Coloured individuals in the sample. The association between presence of the ridge and race is a fascinating result and further investigation is needed in order to confirm and validate this association.

There was a significant difference in the average ages between the individuals with and without the ridge. This coupled with the significant t-test value indicates that the presence of the ridge is associated with age and that the younger the individual, the more likely the individual is to have the ridge present. An explanation for this association may be possible when considering the surrounding structures of the aortic arch where the ridge was observed. The ligamentum arteriosum is found on the adventitial surface of the vessel in the region where the ridge was located. Therefore it is possible that the ridge present in the current study may be a result of the closure of the ductus arteriosus and thus is more frequently observed in younger individuals. This will be further discussed with the histology analysis.

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5.5.3 Histology of the aortic ridge

5.5.3.1 Acid mucopolysaccharides

All ten ridged and non-ridged sections analysed in the aortic ridge sample were positively stained for acid mucopolysaccharides. This is not surprising after the analyses of the histology sample, where similar results were obtained. Both the histology and aortic ridge samples were acquired from the same source and thus a similar explanation may be possible for this abundance of acid mucopolysaccharides. This is that the diet of South Africans, which includes high amounts of animal protein, fat and refined sugars (Steyn, 2007; Voster, 2002), may be responsible for the abundance of acid mucopolysaccharides seen in both the histology sample and the aortic ridge sample.

5.5.3.2 Measurements of the vessel wall

There was no significant difference in the average thickness of the tunica intima between the ridged and non-ridged sections in this sample. This was to be expected as the samples were taken from the same level of the aorta and at non-branching points with no signs of atherosclerotic changes.

There was a significant difference in the average tunica media thickness between the ridged and non-ridged sections. The average tunica media thickness of the non-ridged sections was 1261µ, while the average tunica media thickness of the ridged sections was 1885µ. This result may support the hypothesis that the ridge is a result of the closure of the ductus arteriosus as differences in aorta vessel wall thickness, at the point of the ligamentum arteriosum, have been documented in porcine aorta (Pearson et al., 2008). Pearson et al. (2008) found that the porcine aortic isthmus, where the ligamentum arteriosum is found, was thicker than the descending thoracic aorta. It was also evident that there was a transitional zone between the ligamentum arteriosum and the adventitia and media of the aorta, which included incomplete elastin fibres and increased collagen.

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5.5.3.3 Elastin fragmentation

The elastin fibres of the ridged samples in the present study were considerably fragmented. This is evident from the average elastin grade of 2.90 for the ridged samples compared to the average elastin grade of 1.10 for the non-ridged sample which was significantly different. The grade of 2.90 is similar to the grades of elastin fragmentation at the bifurcation sites. The altered haemodynamics as described earlier at flow separation points is the possible reasoning for the increased elastin fragmentation at the bifurcation sites. Haemodynamic forces are unlikely to be the causative factor at the ridged sample however, as the non-ridged sample which was taken at the same level and very similar site did not less than half the average elastin fragmentation grade.

Yet, once again, there is evidence that elastin fragmentation in the tunica media of the aortic arch may be a result of the ligamentum arteriosum as the elastin fragmentation and increase in collagen observed in this study (Figure 4.56) is similar to those described in porcine aorta by Pearson et al. (2008).

Elastin fragmentation has not been investigated in previous studies on sinutubular or subaortic ridges and therefore no comparison can be made between the ridges described in the present study and the sinutubular or subaortic ridges described previously with regards to elastin fragmentation.

5.5.4 Summary of Aortic ridge findings

There is an association with the presence of the aortic ridge and age as well as a weak association ($p=0.1$) between the presence of the ridge and race. With fewer Coloured and White individuals, a confident association between the presence of the ridge and race is not possible and needs to be investigated further.

The association between the presence of the ridge and age may be explained with a proposed hypothesis that the ridge results from the closure of the ductus arteriosus. This is supported by the more frequent prevalence of the ridge in younger individuals, as well as similar histological features to those previously described in porcine aorta at the point of the ligamentum arteriosum (Pearson et al., 2008). Such histological features include an increased thickness of the vessel wall, fragmentation of the elastic fibres and increased amounts of collagen in the tunica media.

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Although the hypothesis of this aortic ridge being a result of the closure of the ductus arteriosus has been proposed and may have merit, it is important to determine whether this ridge is present in other mammals firstly and then furthermore to determine the effects of HIV on the vessel walls in a similar sample. Thus a comparative anatomy study amongst mammals to determine the presence of the ridge in mammals needs to be investigated as well as the effects of HIV on the histological structure of the aortic vessel wall in order to confirm the hypothesis for the aortic ridge.

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5.6 Limitations of the study

5.6.1 External Validity

The samples from which aortae were investigated were from cadavers of the Human Biology Department, UCT and individuals undergoing forensic autopsy at Salt River Mortuary, Cape Town. As described earlier in this chapter, these samples are skewed for a number of reasons. Due to these factors the generalisation of the results to a broader South African population is limited. The results of this study should therefore only be applied to either cadaver or mortuary populations.

5.6.2 Histological investigation

5.6.2.1 Sample size

The sample size of the histological investigation of the aorta (n=25) and particularly the aortic ridge (n=10) are small and therefore lack statistical power. This was due to only 25 of the mortuary aorta samples being complete and devoid of macroscopic pathology as well as the scope of the Masters degree and the time constraints associated with it.

5.6.2.2 Mortuary data

One of the proposed explanations for the variation in histological structure seen in this sample is the effect of HIV on the vessel wall. The HIV status of the individuals in this sample was not determined during routine forensic autopsy and therefore the HIV status was unknown. Thus no association between HIV status and histological variation seen in the mortuary sample was made.

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5.7 Recommendations for future studies

5.7.1 Variation in the branching pattern of the aorta

The frequency of variation of branching vessels along the total length of the aorta in the UCT cadaver sample was found to be 49%. This is a high frequency of variation when compared to the literature and it would be useful to compare this frequency with other cadaver samples at other universities in South Africa. Future research is need for this comparison and should focus on cadaver samples particularly at universities other than UCT to obtain an indication of the frequency in branching patterns of the idea.

5.7.2 HIV and elastin fragmentation

As discussed, elastin fragmentation has been associated with positive HIV status (Tipping, 2006; Chetty, 2000). With an estimated 5.38 million people in South Africa living with HIV (Statistics South Africa, 2011), HIV must be considered as a potential causative factor for the elastin fragmentation seen in this study. As the HIV status of individuals in this study was unknown, the association between HIV status and elastin fragmentation could not be determined. Future research in this regard should seek to ascertain the association between HIV and histological changes such as elastin fragmentation in the vessel walls of a South African population, possibly including the effects of highly active antiretroviral treatment.

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5.7.3 Acid mucopolysaccharides and diet

Sandhyamani (1999) noted that the Trivandrum population in India ate a diet high in carbohydrates and low in protein and concluded that a large number of these individuals were found to have acid mucopolysaccharides in their blood vessels. It is therefore possible that the high prevalence of acid mucopolysaccharides documented in the Cape Town mortuary sample may be linked to a similar diet. This hypothesis needs further investigation in the future.

5.7.4 Aortic ridge as a result of the closure of the ductus arteriosus

The hypothesis that the ridge results from the closure of the ductus arteriosus has been proposed from the investigations in the current study. This hypothesis is supported by the

frequent prevalence of the ridge in younger individuals, as well as similar histological features to those previously described in porcine aorta at the point of attachment of the ligamentum arteriosum (Pearson et al., 2008). Significant t-test values indicated that the presence of the ridge is associated with age and that the younger the individual, the more likely the individual was to have the ridge present. However, it would greatly increase the significance to incorporate more samples of younger individuals into future research and to investigate the histology of the vessel at the ductus arteriosus in neonates to determine any similarities to those found in the current study. It would also be beneficial make a comparative study to other eutherian fetal mammals as this aortic ridge has now been described in humans and pigs and it would be interesting to see if it was present in other mammals.

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Chapter 6: Conclusion

6.1 Conclusion

The aim of this present study was to document the variation in branching pattern of the aorta in a South African cadaver population as well as document the histological structure of selected regions of the aorta and its main branches in a South African mortuary sample. Thereafter the study aimed to determine any association between histological structure of the vessel wall and the physiological forces acting on the vessel walls.

In keeping with the aims of the project, there were three noteworthy findings which emerged from this study. The first was that there was a high frequency of variation in branching patterns of the aorta documented in the cadaver sample. These included some exceptionally rare branching patterns. Clinically, knowledge of variations in the branching patters of the aorta would be relevant and useful to anatomists, radiologists and head, neck, thoracic and vascular surgeons.

The second result of note was the variety of histological variation of the aorta in the mortuary sample. The most noteworthy of these being, the documented abundance of acid mucopolysaccharides, the increased tunica intima thickness, and the elastin fragmentation of the tunica media at branching sites of the aorta. A possible explanation for the documented abundance of acid mucopolysaccharides may be the link between acid mucopolysaccharides and diet, however this needs further investigation.

It is postulated that altered haemodynamic forces created by turbulent flow at branching sites is an explanation for the increase in the tunica intima thickness. This occurs in order to restore and maintain levels of sheer stress.

As discussed earlier, the increased elastin fragmentation at the branching sites in this sample may be due to a combination of many factors; however the association between altered haemodynamic forces and increased elastin fragmentation seem the most likely cause as there is a marked difference between the elastin fragmentation at branching and non-branching sites. To what extent other factors such as HIV have an effect on the fragmentation of the elastin fibres and other changes to the vessel wall of this sample are yet to be determined.

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Lastly, the identification of and the investigation into the histological structure of an aortic ridge, observed in the majority of the mortuary sample, were noteworthy. This ridge, found on the luminal surface of the aorta at the junction of the aortic arch and descending aorta, to the knowledge of the author of this study, has not been published in the literature and therefore this study is the first that describes such a ridge. Due to the investigations of this study, I propose that the aortic ridge is a result of the changes to the vessel wall associated with the closure of the ductus arteriosus. Is anticipated that a further study including a cohort of individuals from neonates to 11 years would provide evidence of the stages of the closure of the ductuc arteriosus and confirm this hypothesis of the causation of the aortic ridge.

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Appendix 1: Mortuary data recording sheet

1. General Information

WC DR number:

Type of case: Natural / Unnatural COD:

Race:

Sex: Male Female

Age:

2. Aorta

Normal / Fatty Streaks / Plaques / Ulcerations / Calcifications / Haemorrhage / Aneurysm

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Appendix 2: Data collection sheet for aortic branching patterns

Cadaver number:

Aortic arch

- Branches present

- Relative position

- Pathology

Thoracic Aorta

- Branches present:
    - 9 pairs of posterior intercostals arteries
    - Pericardial
    - Bronchial
    - Oesophageal
    - Mediastinal
    - Superior phrenic arteries
    - Subcostal arteries

Some of these branches are difficult to locate and identify, and in many cases had been damaged or removed during student dissection.

- Relative position

- Pathology

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Abdominal Aorta

- Ventral branches present:
  - Coeliac trunk
  - Superior mesenteric artery
  - Inferior mesenteric artery
- Paired branches present:
  - Inferior phrenic arteries
  - Middle suprarenal arteries
  - Renal arteries
  - Testicular/ovarian arteries
  - 4 pairs of lumbar arteries
- Unpaired branch
  - Median sacral artery

Heart

- Left ventricular wall thickness:

- Condition of valves:

- Condition of coronary arteries:

General Pathology

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Appendix 3: Staining methodology

Haematoxylin and Eosin:

Preparation of stain

a) Mayer's Haemalum

Reagents

Haematoxylin	1gm
Potassium aluminium sulphate	50g (anhydrous) / 95g (hydrated)
Sodium iodide	0.2g
Chloral hydrate	50g
Citric acid	0.85g
Distilled water	1000mL

Dissolve haematoxylin in water to 60 degrees Celsius (sunset colour). Add individually the same ingredients in the same order as above. Mix well between each addition. Let solution cool to room temperature overnight and filter before use.

b) 1% Acid Alcohol

Reagents:

70% Alcohol	990mL
Concentrated hydrochloric acid	10mL

In a sufficiently large container, add the acid to the 70% alcohol and mix thoroughly. The generation of fine bubbles is an indicator that the mixing is thorough.

c) Scott's Tap Water

Reagents:

Sodium hydrogen carbonate	3.5g
Magnesium sulphate	20g
Distilled water	1000mL

Pinch of thymol (preservative)

Dissolve salts in distilled water and store at room temperature

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d) Eosin / Phloxine stain

Reagents:

1% Eosin Y, aqueous (Cl 45380)	100mL
1% Phloxine B, aqueous (Cl 45410)	10mL

Mix the two solutions and allow ripening for two weeks. Then dilute 1:1 with distilled water and allow standing in a dark cupboard for a further two weeks.

Staining method:

Dewax sections and bring to water

Stain in Mayer's Haemalum, 10 minutes

Rinse in water

Differentiate in 1% Acid alcohol solution

Rinse in water

Blue in Scott's solution, 2 minutes

Rinse in water

Stain with eosin, 4 minutes

Dehydrate, clear in xylene and mount

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<u>Alcian Blue pH2.5 - Periodic Schiff reactions</u>

Preparation of stain:

a) Alcian Blue

Reagents:

Alcian blue	1g
3% acetic acid	100mL

Dissolve 1g Alcian Blue in 100mL of 3% acetic acid

b) Periodic acid solution

Reagents:

Periodic acid	1g
Distilled water	100mL

Dissolve 1g periodic acid in approximately 50mL of distilled water. Make up to 100mL.

c) Schiff's reagent

Reagents:

Basic Fuschin	1g
Potassium metabisulphite	2g
Con HCL	2mL
Activated charcoal	2g
Distilled water	200mL

Dissolve 1g of basic Fuschin in 200mL of boiling water; remove the flask of boiling water from the Bunsen burner just before adding the basic fuschin. Allow the solution to cool to 50 degrees Celsius, and add 2g of potassium metabisulphite with mixing. Allow to further cool to room temperature, and then add 2ml concentrated HCL. Mix the solution and add 2g activated charcoal and leave overnight in the dark at room temperature. Filter and store in a dark container at 4 degrees Celsius. The solution should be pale or pale yellow.

Staining method:

Dewax sections and bring to water

Stain with Alcian blue solution, 5minutes

Wash in water, then distilled water

Stain with 1% aqueous periodic acid, 5 minutes

Rinse well in water

Stain with Schiff's reagent, 15 minutes

Wash in running tap water, 5-10 minutes

Stain nuclei lightly with haematoxylin solution, ±1 minute

Wash in water

Dehydrate, clear in xylene and mount

<u>Elastin von Gieson's stain</u>

Preparation of stain:

Reagents

a) Elastic stain solution

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5% Alcoholic haematoxylin	5mL
10% Ferric chloride	2mL
Lugol's iodine	2mL

5% Alcoholic haematoxylin: add 5g haematoxylin to approximately 20ml of warm absolute alcohol. Then make up to 100mL with absolute alcohol and leave to ripen for a month before use.

10% Ferric chloride: take 16.5 mL commercial 60% ferric chloride and make up to 100ml with distilled water.

Lugol's iodine: dissolve 4g of potassium iodide in approximately 10mL of distilled water. Add and dissolve 2 g of iodine in this solution and finally make up to 200mL with distilled water

b) Von Gieson solution

Reagents

Saturated picric acid	100ml
1% aqueous acid Fuchsin	10ml
Concentrated hydrochloric acid	0.25ml

Add 100mL saturated picric acid to 10mL 1% aqueous acid Fuschin and boil for 3minutes and allow cooling. Filter and add 0.25mL concentrated hydrochloric acid.

Staining method:

Bring sections to water

Elastic stain solution, 20 minutes

Wash in distilled water

Differentiate in 2% ferric chloride with aid of microscope, rinse in distilled water between

Wash in water

Rinse in 96% alcohol

Wash in distilled water

Counter stain with Von Gieson, 3minutes

Blot dry, dehydrate in absolute alcohol only, clear in xylene and mount

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Appendix 4: Manufacturer and catalogue numbers of the chemicals used in the preparation of the stains

Acetic acid: Associated Chemical Enterprises LTD; A0011FC02500

Acid fuschin: Merck Germany: 650K2624331

Activated charcoal: Hopkin & Williams LTD England; 401571301764

Alcian blue: The British Drug House LTD; 34089

Basic fuschin: Merck SA; SAAR 250 15 00 DC

Chloral hydrate: Merck SA; 159 15 00 EM

Citric acid: Merck SA; 100247

Concentrated hydrochloric acid: Holpro Analytics LTD; 74174

Eosin yellowish: Merck SA; 218 60 00 CB

Haematoxylin: Merck SA; 282 20 00 CB

Iodine: Merck SA: SAAR 311 28 00 EM

Magnesium sulphate: Merck SA; SAAR 412400 EM

Phloxine B: Merck Germany; K28 063 226

Picric acid: The British Drug House LTD; 10271

Potassium aluminium: Merck SA; 111 80 00 EM

Potassium iodine: Holpro Analytics LTD; 77202

Potassium metabisulphate May & Becker LTD Dagenham England; P137/18/66

Sodium hydrogen carbonate: Merck SA; 582 28 20 EM

Sodium iodine: The British Drug House LTD; 0548050852600

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